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THE UNIVERSITY OF ALBERTA

Odorous Aldehydes Arising From Reactions of Amino Acids with Disinfectants in Drinking Water

by

Alina J. Gac

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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DEGREE OF MASTER OF SCIENCE

IN

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DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA

(Fall, 1988)

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Date: Oct 8, 1992

ABSTRACT

A search for the cause of Edmonton's spring time taste and odour problem occurring in two successive years, implicated four low molecular weight aldehydes; isobutyraldehyde, 2-methyl butyraldehyde, isovaleraldehyde and phenylacetaldehyde. These chemicals have odour thresholds ranging from 0.15 to 12.5 µg/L. Although these aldehydes have been reported found in drinking water, not much attention has been given to their occurrence. With the exception of a passing reference to odour contributions from phenylacetaldehyde as one of several odorous metabolites of fungi and blue green algae, these compounds have not been reported as a cause of taste and odour problems in water. Observations of more intense odour in treated than in raw water suggested the possibility of these compounds being produced in the treatment process.

One explanation for the experience in Edmonton is that the odorous aldehydes could arise as oxidation products of corresponding amino acids through their oxidation by disinfecting agents. This research studied the reactions of the amino acids: valine, leucine, isoleucine and phenylalanine with four disinfection agents: chlorine, monochloramine, chlorine dioxide and ozone, with respect to aldehyde formation. Quantitative analysis of the produced odorous aldehydes, with sufficient sensitivity to detect these compounds at their threshold odour levels, allowed the study of their yield under relevant water treatment conditions. The reaction parameters evaluated included: pH, temperature, reaction time and oxidant/amino acids molar ratio.

This research demonstrated that chlorine was very effective in producing aldehydes from their corresponding amino acids. Temperature, molar ratio and reaction time have major effects on aldehyde yields. The effect of pH was found to be negligible in the range from 6 to 10. Monochloramine was also effective in conversion of amino acids to aldehydes but longer times and higher dosages were required to maximize aldehyde yields which also strongly depend on pH. Both, chlorine and monochloramine perform these reactions at concentrations and conditions which are encountered in

conventional water treatment practice. Ozone provided very low aldehyde yields while chlorine dioxide did not appear to produce aldehydes in any substantial quantity.

Given that the odorous aldehydes are formed from amino acids which are ubiquitous and unavoidable dissolved organic species in surface waters, there is a great potential for taste and odour problems arising from this souree wherever surface water is utilized as a raw water source.

To minimize the occurrence of this problem in drinking water, low concentrations of amino acids, whether free or in peptides, should be obtained before chlorination or chloramination. If chlorine dioxide is used, the degree of chlorine contamination should be strictly limited.

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1. INTRODUCTION

1.1 Background for the research.

A severe taste and odour problem occurred in Edmonton's drinking water during the springs of 1985 and 1986. A subsequent investigation (Hrudey, 1986) suggested possible causes and provided the basis for an experimental investigation.

Edmonton's drinking water is drawn from the North Saskatchewan River at two treatment plants. One is located upstream of the city and the other is located in the city centre, downstream of 85 storm sewer outfalls. Taste and odour problems had in the past occurred together with spring runoff. These problems were associated with urban runoff (Hrudey, 1986; Walker, 1986) and occurred only in the city centre plant. However, the 1985 and 1986 incidents were substantially different in their circumstances and character. Available evidence suggested that the taste and odour were related to natural rural runoff (Hrudey, 1986). Geosmin and possibly methylisoborneol were found at very low levels in raw and treated water in 1986. However, the threshold odour number of the treated water was much higher than that of the raw water which implied the formation of some odorous by-products during water treatment.

Chemical analysis of treated water revealed the presence of four aldehydes; isovaleraldehyde (3-methyl butanal), 2-methyl butyraldehyde (2-methyl butanal), isobutyraldehyde (2-methyl propanal) and phenylacetaldehyde. The food science literature indicates that these aldehydes are very odorous with threshold odour concentrations in the range of µg/L. Their presence in treated water raised the question of their origin. Hrudey (1986) proposed that these aldehydes were formed by chemical oxidation of amino acids whose concentrations in raw water are expected to rise during rural runoff.

Documentation from the literature was found to support this theory. However, no report on formation of isobutyraldehyde 2-

methyl butyraldehyde and isovaleraldehyde from the corresponding amino acids; valine, isoleucine and leucine during chlorination of drinking water was found in the literature. Moreover, their presence has not been reported as the case of specific taste and odour incidents in municipal drinking water. Phenylacetaldehyde has been shown (Le Cloirec and Martin, 1985) to be a by-product of chlorine oxidation of phenylalanine. As well, Kikuchi et al. (1983) identified phenylacetaldehyde as one of several odorous metabolites of certain fungi which, they suggest, may contribute to taste and odour problems in drinking water.

1.2 Research objectives.

Given the hypothesis that odorous aldehydes could be produced by reaction with drinking water disinfectants, specific research questions are apparent. The objectives of this research were:

1. to evaluate the formation of isobutyraldehyde, 2-methyl butyraldehyde, isovaleraldehyde and phenylacetaldehyde under water treatment conditions from the corresponding amino acids; valine, leucine, isoleucine and phenylalanine reacting with the oxidants commonly used for disinfection:

    - chlorine
    - monochloramine
    - chlorine dioxide
    - ozone

2. to investigate the effects of reaction conditions on the formation of aldehydes, specifically:

    - reaction time
    - reaction temperature
    - oxidant/amino acid molar ratio
    - pH

To achieve these objectives the research was pursued in three stages. First, the literature was reviewed in detail to provide the background for the experimental part of the research. Secondly, the methods and procedures for quantitative determination of aldehydes were developed. Last, specific experiments designed to meet the foregoing objectives were designed and performed.

2. LITERATURE REVIEW

2.1 Sources and character of odour problems in drinking water.

Taste and odour causing compounds are becoming recognized more frequently as an important category of organic micropollutants in water. Offensive tastes and odours in drinking water cause public dissatisfaction and concern for the implied poor water quality (Bartels et al., 1986) and the potential presence of toxic substances (Mallevialle and Suffet, 1987). This concern is reflected in expanding research activities on taste and odour problems which are resulting in significant progress toward recognition, understanding and solution of taste and odour problems.

Many different odorous compounds have been found in waters. These substances can be present in raw water sources, they can be formed during water treatment, particularly by disinfection processes, or they can develop in distribution systems.

Substances present in raw waters can be divided into two groups; naturally occurring and man-made. Among naturally occurring odorous chemicals, there are many metabolites produced by various aquatic organisms such as bacteria, algae, fungi and zooplankton. Mallevialle and Suffet (1987) and Lin (1976) supply comprehensive lists of organisms producing odorous compounds. The biologically produced substances, geosmin and 2-methylisoborneol (MIB) are particularly noteworthy. Geosmin was isolated and identified in 1965 by Gerber and Lechevalier while MIB was isolated in 1969 by Medsker and co-workers and by Gerber. These compounds are often discussed in the literature because of their frequent occurrence, their extremely low threshold odour concentrations and the difficulties which they pose to conventional water treatment processes.

Since the initial identification of those metabolites many other substances have been reported as odorous products of different microorganisms. Among those often cited are mucidone, mercaptans,

alcohols, fatty acids, esters, aldehydes, ketones, acids and many others. Figure 2.1 provides chemical structures of these compounds.

Naturally occurring inorganics such as iron, manganese and sulfur compounds can account for odour in ground water supplies (AWWA, 1976).

Anthropogenic sources of taste and odour include various chemicals arising from industrial wastes, agricultural activities, municipal wastewater and runoff from rural and urban areas. Chlorinated phenols and hydrocarbons have contributed to taste and odour in drinking water.

Another group of odorous compounds may be created during water treatment. Compounds present in raw water can be modified by physical, chemical and biological processes occurring within the water treatment plant as well as in the distribution system. Mallevialle and Suffet (1987) observe that the oxidative processes of chlorination and ozonation will be the most important processes related to taste and odour problems.

Various taste and odour causing compounds develop as a consequence of chlorination. Chlorine can react with naturally occurring organics in two basic ways. First, it can oxidize organic compounds to form new chemicals by accepting electrons. Second, chlorine can substitute onto the raw water organics to create chlorinated organics (Mallevialle and Suffet, 1987).

Chlorination of phenol and its homologs produces chlorinated phenols which are more odorous than the parent compounds. Chlorination can also accentuate the odour of metabolic origin (AWWA, 1976). Chlorine and chloramines also contribute tastes and odours themselves (Krasner and Barrett, 1984).

Although ozone and chlorine dioxide have often been cited as efficient agents in controlling off-odours (Lazary et al., 1986, Mallevialle and Suffet, 1987), they can also be responsible for intermediate reaction products impairing the aroma of water. Ozonation may lead to the formation of aliphatic and aromatic aldehydes, same of which are odorous. Information on odours arising from chlorine dioxide is less well documented. However, chlorine dioxide does have an advantage over chlorine of producing

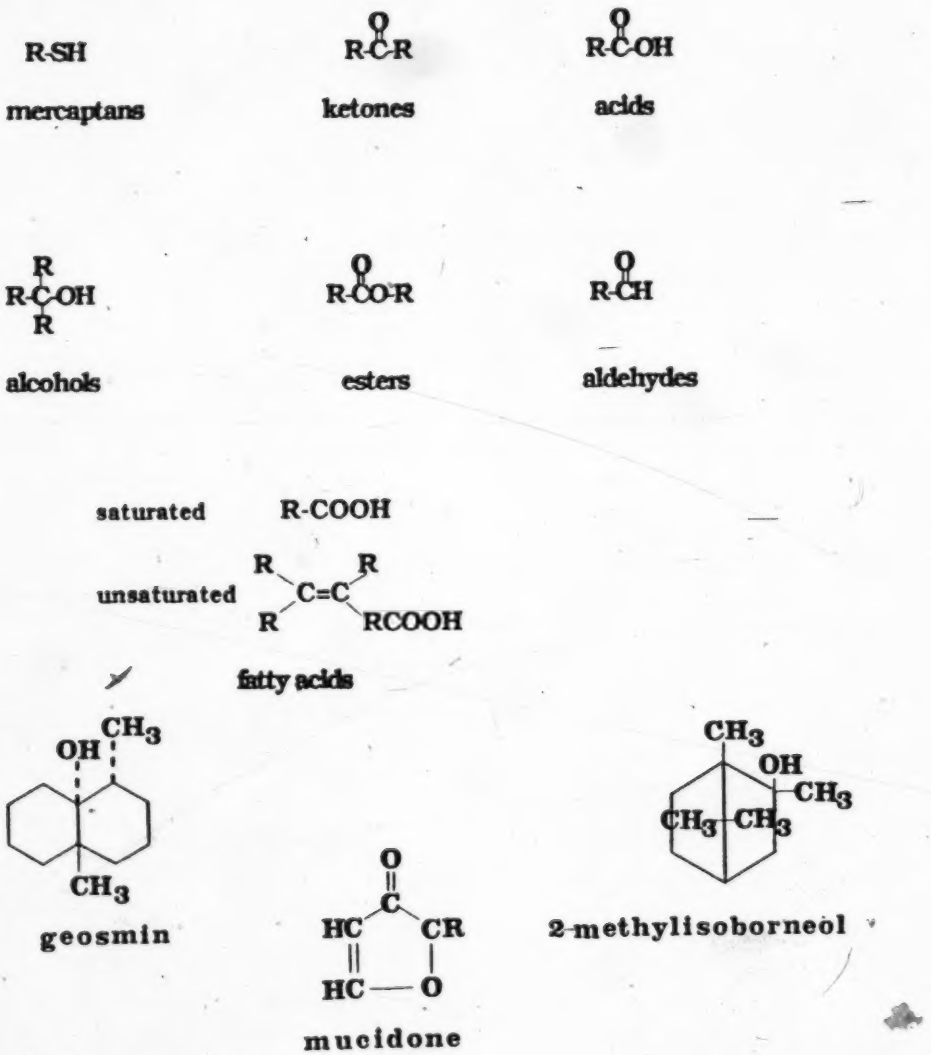


Figure 2.1 Structural formulas of odorous compounds often encounter in drinking waters

considerably fewer chlorinated products. Consequently, it does not lead to as many chlorinous tastes and odours (Mallevialle and Suffet, 1987).

Despite the progress in taste and odour research in recent years, many causes of offensive tastes and odours in drinking water remain unidentified. These problems reflect the need for further study in this field. Identification and characterization of these problems are necessary to achieve effective control.

2.2 Aldehydes as odour causing compounds.

Four aldehydes were identified as odorous compounds in drinking water during the 1985 and 1986 taste and odour incidents in Edmonton; isobutyraldehyde (2-methyl propanal), 2-methyl butyraldehyde (2-methyl butanal), isovaleraldehyde (3-methyl butanal) and phenylacetaldehyde (Hrudey, 1986). These aldehydes have been found in drinking waters, however, not much attention has been given to their occurrence.

2.2.1 Contribution of aldehydes to odour in drinking water.

The potential contribution of these aldehydes to odour in drinking water had not been clearly recognized in literature.

Amoore (1986) has worked on classification and characterization of the primary odours. He included isobutyraldehyde and isovaleraldehyde in the list of 90 chemicals that should be evaluated as odour causing in drinking water. Isobutyraldehyde was also included in the list of 101 various chemicals of industrial origin that may be encountered in drinking water and cause odour problem (Mallevialle and Suffet, 1987).

Kikuchi et al. (1983) reported isolating phenylacetaldehyde as one of several odorous metabolites of various fungi which they proposed as contributors to odours in public water supplies. These references do not cite any examples where these chemicals have

caused problems. No mention of 2-methyl butyraldehyde as a potential cause of odour in drinking water has been found in the reviewed literature.

2.2.2 Chemical and physical properties of the odorous aldehydes.

Isobutyraldehyde, 2-methyl butyraldehyde and isovaleraldehyde are aliphatic, branched (iso type), low molecular weight aldehydes. Phenylacetaldehyde is an aromatic aldehyde. The structural formula of each and their alternate names are presented in Figure 2.2.

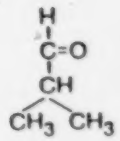
The chemical and physical characteristics of the four aldehydes are summarized in the form of a table and are presented in Appendix A.

These aldehydes are difficult to analyse and they could be easily missed unless the analyst is specifically seeking them. They are relatively volatile and susceptible to further oxidation or polymerization. Identification by conventional MS operation may be a problem. Most of the fragment ions for these compounds arise below a mass/charge of 45, with the most characteristic fragment at mass/charge of 29. Common practice in many analytical laboratories is not to monitor fragments below mass 45. This practice would result in substantially reduced sensitivity on total ion chromatogram and impaired ability at compound identification. These factors may explain the limited number of reports of the aliphatic aldehydes in water (Hrudey, 1988).

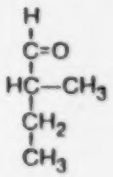
2.2.3 Odour characteristics of the aldehydes.

The importance of the odour characteristics of these aldehydes has been recognized in the food science literature. They are responsible for aroma and flavour of many foodstuffs. A wide variety of food science literature supplies characteristics of their aroma and odour intensity.

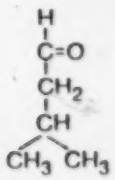
2-methyl propanal or isobutyraldehyde (also isobutyl aldehyde, isobutyric aldehyde), C_4H_8O, MW = 72.10;



2-methyl butyraldehyde or 2-methyl butanal, $C_5H_{10}O$, MW = 86.13;



isovaleraldehyde or 3-methyl butanal (also isovaleral, isovaleric aldehyde), $C_5H_{10}O$, MW = 86.13;



phenylacetaldehyde or benzeneacetaldehyde (also alpha-Tolualdehyde), C_8H_8O, MW = 120.14

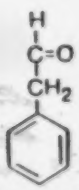


Figure 2.2 Structural formulas of aldehydes.

Published threshold odour concentrations in water for these aldehydes are summarized in Table 2.1. These data show the aldehydes to be extremely odorous.

Isovaleraldehyde and isobutyraldehyde exhibit odour which resembles malt and they are indeed the principal volatile carbonyl compounds in barley malt. Amoore et al. (1976) have proposed isobutyraldehyde as responsible for the primary malty odour. The primary odorants are those for which the sense of smell has an especially high sensitivity. These refer to a limited numbers of discrete "primary odour" sensations which can be combined in different proportions to give a tremendous range of distinguishable odours (Amoore and Buttery, 1978).

A very high sensitivity of human beings for detecting these particular aldehydes is, according to Amoore et al. (1975), associated with the fact that each of them is derived from an amino acid indispensable in the human diet. Hence, their intense odour may be an innate mechanism for detecting the presence of required nutrients by their volatile degradation products.

Amoore et al. (1975) conducted olfactory experiments on 14 aliphatic, branched, short chain aldehydes. They showed that, though extremely odorous, 2 methyl butyraldehyde, isovaleraldehyde and isobutyraldehyde are subject to anosmia. Anosmia refers to the loss or impairment of the sense of smell. This effect is most pronounced with isobutyraldehyde. Amoore et al (1975) studied the response of 225 panelists to these aldehydes and found that 36% of their group was insensitive to isobutyraldehyde. Consequently, differences in water odour intensity perception by different people may arise because of the high variability in population sensitivity to these aldehydes. The anosmic effect of these three aldehydes is summarized in Table 2.2. People with anosmia to isobutyraldehyde require about 500 times higher concentration for detection. The effect is less pronounced for isovaleraldehyde (about 9 times higher concentration required) and 2-methyl butyraldehyde (about 17 times higher concentration required).

A detailed analysis of the odour characteristics of aldehydes performed by Guadagni et al. (1963) showed that the odour intensity

Table 2.1 Aldehyde detection threshold odour levels in water.

compound	threshold level µg/L	reference
isobutyraldehyde	0.9	Guadagni et al. 1963
	1.0	Guadagni et al. 1972
	2.3	Amoore et al. 1976
	1.8	Amoore 1986
isovaleraldehyde	0.15	Guadagni et al. 1963
	0.2	Guadagni et al. 1972
	2.0	Amoore et al. 1976
2-methyl butyraldehyde	12.5	Amoore et al. 1976
phenylacetaldehyde	4.0	Buttery et al. 1971
	4.0	Guadagni et al. 1972

Table 2.2 Anosmic and normal detection threshold odour levels of aldehydes (Amoore et al., 1975).

	threshold odour concentration water (µg/L)	
compound	normal	anosmic
isobutyraldehyde	2.3	1190
isovaleraldehyde	2.02	17.8
2-methyl butyraldehyde	12.5	212

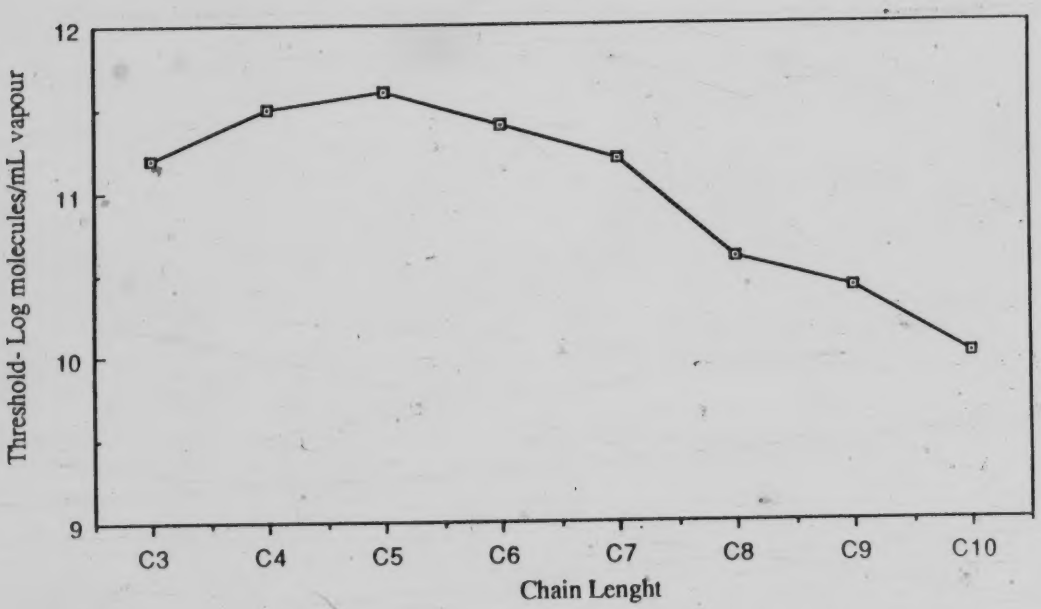


Figure 2.3 Relationship between chain length of a series of saturated aldehydes and threshold concentration (adapted from Guadagni et al., 1963).

of aldehydes increases with increasing carbon chain in the range of C_3-C_{10} (Figure 2.3). Guadagni et al. (1963) also noted that the branched, short chain aldehydes had much lower odour threshold concentration than the corresponding straight chain compounds.

The odour character of the aldehydes is complex which may lead to different perception by different people. This circumstance is further complicated by a change in perception of the odour character from high to very dilute concentrations.

The odour description of isovaleraldehyde presented by Cox (1975) emphasizes terms such as pungent and apple-like. Dravnieks (1985) using a panel of 140 evaluators and a high concentration of isovaleraldehyde (20g/L) reported terms with the frequency indicated: fruity, other than citrus (4.55%), fragrant (7.58%), aromatic (11.51%), almond (6.14%), woody, resinous (13.97), sweet (8.85), yeasty (4.77%), dirty linen (4.42%), stale (6.5%), musty, earthy moldy (12.67%), burnt smoky (6.59%), chemical (10.11%), sharp, pungent, acid (18.71%) sour, vinegar (15.82%), oily, fatty (11.15%), paint (6.86%), rancid (16.18%), sweaty (16.32%), sulfidic (5.23%), animal (5.79%), sewer (5.68%), putrid, foul, decayed (17.55%), fecal, manure like (6.13%), cadaverous, dead animal (4.17%), sickening (26%), dry, powdery (4.42%), light (4.73%), heavy (22.38%), warm (10.21%). Another 88 descriptors were used by at least one of the 140 evaluators. Metoda (1979) describes the odour of isovaleraldehyde as chocolaty.

The odour of isobutyraldehyde has been described as malty (Amoore, 1976), apple-like (Metoda, 1979), sweet and pungent (Cox, 1975), sweet and ester (Verschueren, 1983) and pungent (Merck Index, 1983).

Similar terms were used to described the odour of 2-methyl butyraldehyde. They were: apple- or malt-like (Metoda, 1979). During the course of this research, the odour character of these two aldehydes has been observed to be very similar, however, they can be readily distinguished.

The odour description of phenylacetaldehyde is more uniform and is characterized by several sources (Cox, 1975, Metoda, 1979,

Kato et al., 1971) as hyacinth-like and lilac. Le Cloirec and Martin (1985) used the term "jasmine".

The odour complexity of a single compound can be further increased in a mixture of chemicals. Baker (1963) performed a study on the effect on odour responses to some binary and higher order mixtures of organic chemicals. The results showed that in binary mixture: 1) nonadditive odour behaviour frequently occurred, 2) strong synergism was shown for many organic pairs, 3) odour response was not a monotonically increasing function of concentration.

The complexity of the aroma of a drinking water containing a mixture of odorous aldehydes was reflected in the many different terms used to characterize its aroma. With regard to the treated water, a panel of assessors from the department of Foods and Nutrition at the University of Alberta, described the odour with a wide range of terms including: chlorine, earthy, stale, disinfectant, bitter, ammonia, organic, muddy, and bleach-like. Flavour descriptors were: stale, earthy, organic, muddy metallic, chemical, marshy, moldy, musty, sloughy, sewagey, decayed plant-like and oily. A mixture of these odorous chemicals, together with chloramines would be expected to cause a wide range of responses by water consumers. High variability in the sensitivity to and perception of aroma may account for certain people not noticing off-odour in water that the majority finds objectionable.

2.3 Occurrence of aldehydes in drinking water.

The occurrence of aldehydes in drinking water has been reported among many other trace organics identified by sensitive organic analytical techniques.

Dowty et al. (1975) reported trace levels of isovaleraldehyde in New Orleans drinking water where chloramines were used as disinfectants. Coleman et al. (1976) had performed the trace organic survey for five American cities and reported that isobutyraldehyde and isovaleraldehyde were found in drinking water from all five cities but no concentrations were given. Stevens (1982) reported the presence of several aldehydes in drinking water including isobutyraldehyde and isovaleraldehyde in both the raw and chlorine dioxide treated water. However, the aldehyde concentrations in the treated water were substantially greater. Trussell (1986) included isobutyraldehyde and 2-methyl butyraldehyde in a list of 64 trace organics identified in treated water.

The concentration of aldehydes in Edmonton's drinking water during the most intense odour in 1986 was reported to be: 54 µg/l for isobutyraldehyde, 46 µg/l for isovaleraldehyde and 134 µg/l for 2-methyl butyraldehyde. However, these results may not represent the actual concentrations of these compounds during the peak odour incident because the samples were analyzed after 3 months of refrigerated, dark storage (Hrudey, 1986).

In addition to these aldehydes, there was evidence of phenylacetaldehyde at µg/L levels in extractable analyses of both raw and treated water (Envirotest, 1986). As well, Alberta Environment provided estimates of 0.5 to 1.0 µg/L and 10 to 50 µg/L of this compound in two treated samples collected during the peak of the 1986 odour incident in Edmonton (Hrudey, 1986).

Many linear and other unidentified aldehydes were reported found in ozonated waters by different researchers (Sievers et al., 1977, Zoeteman et al, 1982, Van Hoof et al., 1985, Rice and Gomez-Taylor, 1986, Mallevialle and Suffet, 1987). Linear, low molecular weight aldehydes (C_1-C_3) have been demonstrated as ozonation by-products and found in concentrations of 0.13 µmol/L in treated

water by Van Hoof et al. (1986). Mallevialle and Suffet (1987) presented lists of organic compounds detected in drinking water during a two year study conducted to evaluate process efficiencies for taste and odour removal by the specific plants. The aldehydes identified are summarized in Table 2.3. The formation of these aldehydes led to the development of fruity, fragrant and orange-like odours when the concentration of aldehydes reached the level of about 0.1 mg/L.

Garrison (1976) compiled a list of organic compounds identified in various waters that had been reported in the literature. The list includes unspecified aldehydes: 1 aromatic and 5 aliphatic aldehydes found in raw water and 5 aromatic and 37 aliphatic aldehydes found in drinking water.

2.4 Source of aldehydes in waters.

The higher concentrations of aldehydes reported in finished water than in raw water (Dowty et al., 1975, Garrison,1976, Stevens, 1982, Hrudey, 1986) implies that aldehydes are formed during water treatment processes. However, it is also known that these compounds are present in raw waters presumably as natural degradation products of amino acids (Hrudey, 1986) and as metabolites of some species of fungi (Kikuchi et al., 1983).

2.4.1 Biological sources.

The formation of the odorous aldehydes through biological activities has been documented mainly in the food science literature as the products of enzymatic reactions of certain microorganisms on amino acids. A fermenting bacteria *Streptococcus lactis*, for example, has been responsible for development of malty odour in dairy products by forming isovaleraldehyde and isobutyraldehyde from corresponding amino acids (Braun et al., 1982). A number of strains of yeasts were shown (Lee and Richard, 1984) to have the ability to

Table 2.3 Aldehydes identified by GC/MS in water at Morsag Plant in France (from Mallevialle and Suffet, 1987).

compound	occurrence
1-Hexanal	18
1-Heptanal	1
1-Octanal	26
1-Nonanal	257
1-Decanal	262
1-Undecanal	18
1-Dodecanal	20
2-Dodecanal	5
C14 Aldehyde (1)	5
Benzyl-acetaldehyde (1)	9
C9 Aldehyde (1)	1
C9 Aldehyde (2)	3
C10 Aldehyde (1)	5
C12 Aldehyde (1)	9
C14 Aldehyde (1)	4
C13 Aldehyde (1)	2
C13 Aldehyde (2)	4
C15 Aldehyde (1)	3
C9 Aldehyde (3)	2
Hexanal(1)	6
Heptanal (1)	11
Decanal (1)	1
Heptanal (1)	10
Octanal (1)	12
Undecanal (1)	3
Heptanal (2)	10
Octanal (2)	32
Heptanal (3)	20
Octanal (3)	4

A number following a compound indicates various isomers.

produce phenylacetaldehyde from phenylalanine. Bacterial activity on leucine in colon results in production of isovaleraldehyde (Kubow et al., 1981).

The formation of aldehydes from amino acids in the aquatic environment was studied by Nieder and Hager (1985). They report that bromoperoxidase, an enzyme of many marine organisms (for their study obtained from the algae *Penicillus capitus*), converts valine and peptide valylvaline to isobutyrnitrile and isobutyraldehyde, while alanine is converted to acetonitrile and acetaldehyde. The authors believe that this enzyme plays a major role in the formation of halometabolites, nitriles and aldehydes in the marine environment.

As mentioned previously, Kikuchi et al. (1983) found that phenylacetaldehyde as well as benzaldehyde were among odorous metabolite products of fungi *Chaetonium, Penicillium, Robillaria* and *Botrytis cinerea* present in water reservoirs.

A number of various aldehydes can be formed from different organic precursors by bacterial degradation. Aldehydes are intermediate as well as end products of biodegradation of n-alkanes, unsubstituted aromatics and alkylbenzenes (Linden and Thijsse, 1965).

Collins and Kalnins (1965) isolated odorous acetaldehyde, furfuraldehyde, n-heptanal and valeraldehyde from the flagellated algal species *Synudra petersenii*, which, they say, contribute to water taste and odour impairment by this organism. Mallevialle and Suffet (1987) report the works of Kikuchi et al. (1972, 1974) who identified the fishy smelling n-hexanal and n-heptanal in cultures of a diatom *Synedra rumpens* synthesizing fatty acids. Juttner (1983) presented a list of many odorous products of algae including formaldehyde and acetaldehyde as metabolites of *Chlamydomonas globosa*, and formaldehyde, pentanal and hexanal as metabolites of *Cryptomonas ovata*.

2.4.2 Non-biological chemical reactions producing aldehydes.

Formation of various aldehydes by chemical oxidation of a variety of organic substrates has been discussed in the literature. Stevens (1982) and Rice and Gomez-Tylor (1986) state that chlorine dioxide will produce these chemicals from compounds naturally occurring in waters. Stevens suggested alkenes and amines as possible precursors to the observed aldehydes. Aldehydes have been shown to be produced as ozonation by-products from different precursors, including humic and fulvic acids (Rice and Gomez-Taylor, 1986, Van Hoof et al. 1985), fatty acids (Glaze, 1986) unsaturated aliphatic compounds (Fielding, 1986), alkanes, alcohols and other aldehydes (Falk, 1976). Chlorination of humic and fulvic substances also results in aldehydes formation (Rice and Gomez-Taylor, 1986).

Observations on the source of the malty odour in foods, led Hrudey (1986) to recognize that the odorous aldehydes found in Edmonton's drinking water could be the oxidation products of specific amino acids. Amoore et al. (1976) reported that the amino acids; valine, leucine and isoleucine thermally undergo Strecker degradation to produce corresponding aldehydes; isobutyraldehyde, isovaleraldehyde and 2-methyl butyraldehyde as presented in Figure 2.4.

Many other researchers including Bailey et al. (1962), Rooney et al. (1967), Salem et al. (1967), Lipparini and Cavana (1978), Eichner and Ciner-Dorule (1981), Seck and Crouzet (1982) have shown the formation of these aldehydes through thermal degradation. Phenylacetaldehyde production from phenylalanine through the same mechanism (Figure 2.4) has also been well documented (Rooney et al., 1967, Salem et al.,1967, Finot et al., 1967, Kato et al., 1971, Brueckner and Misselhorn, 1977, Seck and Crouzet, 1982).

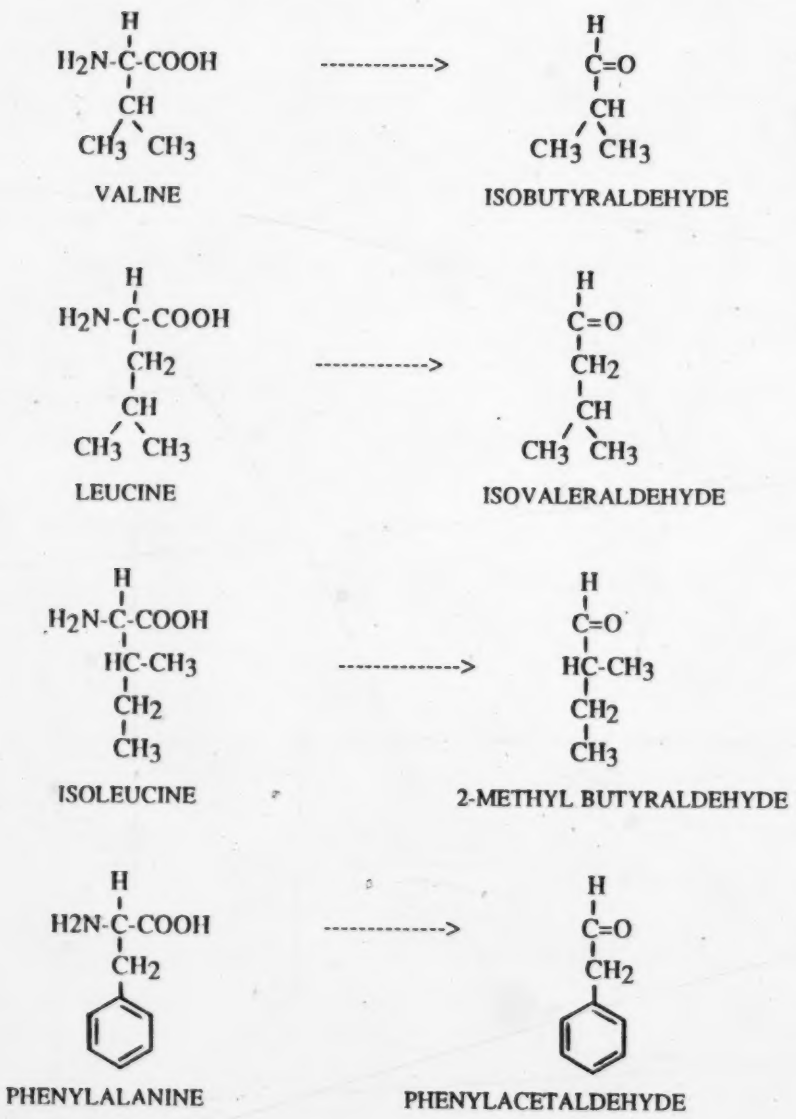


Figure 2.4 Conversion of amino acids to aldehydes

2.5 Amino acids as precursors to aldehydes.

The possibility of amino acids being precursors for aldehydes as a result of chemical oxidation is well documented in the literature. Chlorine and its derivatives and chlorine dioxide are oxidants whose interactions with amino acids have been reported to produce aldehydes. These interactions are relevant because of the chlorine demand created in water by amino acids (Le Cloirec and Martin, 1985, Ayotte and Gray, 1985) and because of the possible formation of by-products which impair the quality of drinking water.

2.5.1 Presence of amino acids in water supplies.

Amino acids are ubiquitous constituents of the environment (Hutchinson, 1957). Their relative abundance in natural waters makes them of particular interest as reactive precursors for disinfectants.

The concentrations of total, free and individual amino acids reported in the literature vary greatly depending on many environmental factors affecting waters. According to Baker et al. (1975) differences in their abundance depend upon the environmental stress, organic load and the degradative oxidation within waters.

Ram and Morris (1982) report that amino acids were found in raw water supplies in concentrations of 5 to 2000 µg/L either in the free state or combined as peptides, nucleic acid, purines, pyridines and proteins. Thurman (1986) reports a range for total amino acids of 50 to 1000 µg/L for rivers and 300 to 6000 µg/L for eutrophic lakes. Free amino acids and peptides averaged 76 and 172 µg/L respectively in 15 American lakes (Hutchinson 1957). Gardner and Lee (1973) identified several amino acids in water from Lake Mendota. Concentrations of individual amino acids were approximately 20 µg/L.

Waters of the Mackenzie River drainage basin in Canada showed the presence of total amino acids in concentrations of 12 to 299 µg/L (Peake et al., 1972). The study of pristine mountain stream waters in the Marmot Creek system in Alberta showed the presence of free amino acids ranging from 0.02 to 2 µg/L. Specifically, phenylalanine concentration varied from 0.04 to 0.18 µg/L, isoleucine from 0.02 to 0.09 µg/L and valine from 0.05 to .23 µg/L depending on the season (Telang et al. 1982). A similar study conducted on waters of the Red Deer River basin showed that amino acid levels were substantially higher than those in the Marmot Creek Basin, and varied from 0.8 to 23.1 µg/L for free amino acids and 1.9 to 142 µg/L for combined amino acids. A different seasonal variation was observed in distribution of amino acids. Their levels in April, for example, increased as much as five fold over the seasonal average of about 5 µg/L. The concentrations of valine, leucine, isoleucine and phenylalanine in the free state ranged from; valine 0.02-1.0 µg/L, isoleucine 0.05-0.75 µg/L, leucine 0.01-1.1 µg/L and phenylalanine 0.01-1.5 µg/L.

Analysis of the North Saskatchewan River water from March 1987 showed the concentrations of free valine, isoleucine, leucine and phenylalanine to be 3.2 µg/L, 1.0 µg/L, 1.2 µg/L and 0.3 µg/L respectively (Daignault, 1987). No data on concentrations of specific nitrogenous compounds in the raw water during the 1985 and 1986 odour events in Edmonton were available. However, the peak of odour intensity corresponded closely with the peaking of dissolved organic carbon (DOC) in the raw water (Hrudey, 1986). A single sample of treated water at the time of peak odour intensity had a total Kjeldahl nitrogen level of 1.45 mg/L which is abnormally high for the North Saskatchewan River.

An increase in natural DOC levels will likely result in an increase in amino acid levels. Thurman (1986) reported that amino acids accounted for 1-3% of the total dissolved carbon for an average river with DOC of 5 mg/L. The distribution of amino acids in natural waters as a percentage of DOC is presented in Figure 2.5 adapted from Thurman (1986).

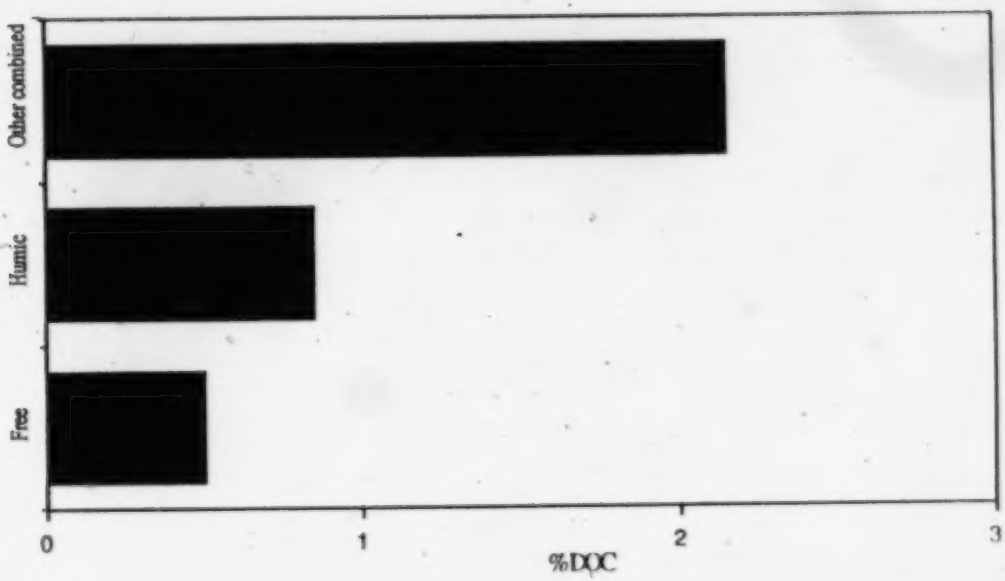


Figure 2.5 Histogram of free and combined amino acids in natural waters (adapted from Thurman, 1986).

Levels of amino acids have been shown to actually increase during water treatment. Le Cloirec and Martin (1985) found that their presence is modified by water treatment processes when studying the occurrence and distribution of amino acids during the production of drinking water. They reported that an increasing concentration of amino acids developed during prechlorination of water which, they explained, resulted from hydrolysis or depolymerization of proteins and peptides found in untreated water, They also indicate that the release of amino acids might occur after filtration perhaps due to microbial activity on filters. Flocculation-decantation does not affect free amino acids concentrations (Rice and Gomez-Taylor, 1986, Le Cloirec and Martin, 1985).

The foregoing studies suggest that amino acids are not removed during water treatment and can be present at a final disinfection step.

Given the possibility of very odorous aldehydes being produced by reaction between common disinfectant chemicals and the ubiquitous amino acids, there is a great potential for odour problems from this source. Because of this potential it is very important to fully understand the interactions of amino acids with oxidative chemicals.

2.5.2 Oxidation of amino acids by disinfecting agents.

Amino acids could be oxidized by any of the common disinfectant chemicals. Detailed review of published reactions of amino acids with chlorine, chlorine dioxide, monochloramine and ozone follows.

2.5.2.1 Reactions of amino acids with chlorine.

The interactions of chlorine and its derivatives (HOCl, OCl^-) with amino acids in an aqueous solution have been thoroughly studied and well documented.

First available records of the studies, as mentioned by Dakin (1916), date from 1857. Since that time a number of publications have reported the reaction pathways, products and kinetics. However, the researchers are divided over exactly what chemicals are produced and the conditions influencing their formation. Generally however, it is well documented that chlorine and its derivatives react with amino acids forming two major products; aldehydes and nitriles. The presence of other oxidation products such as carboxylic acids and chlorinated amino acids has also been reported, depending on the reaction conditions.

The work of Langheld (1909) is often cited. He was the first to note that α-amino acids react with hypochlorous acid salts to form monochlorinated and dichlorinated derivatives which subsequently can decompose forming aldehydes, ketones or acids (after Stanbro and Smith, 1979). The reaction pathway, later confirmed by several researchers, is presented in Figure 2.6.

According to Stanbro and Smith (1979), the decomposition of chloroamino acids is affected by the nature of the α-amino group. If the group is primary, then aldehydes or ketones, NH_3, carbonic acid and sodium chloride are formed. If the group is secondary the same products are obtained except that the corresponding amine replaces ammonia as products.

Fox and Bullock (1951), proposed a mechanism for the decomposition of N-chloro-α-amino acids. Their mechanism, presented in Figure 2.7, shows the importance of pH on the product formation.

Both of the foregoing works proposed monochloroamino acids as the intermediate formation products; and nitriles were not considered as the reaction products.

Dakin (1916), Wright (1926) and Pereira et al. (1973) have studied the decomposition of N,N-dichloro-α-amino acids. Their results indicate rapid formation of CO_2, chloride and corresponding nitrile compounds according to the reaction presented in Figure 2.8 (Dakin, 1916). This reaction scheme indicates that some researchers have not differentiated between the decomposition of N-chloroalpha-amino acids and N,N-dichloroalpha-amino acids.

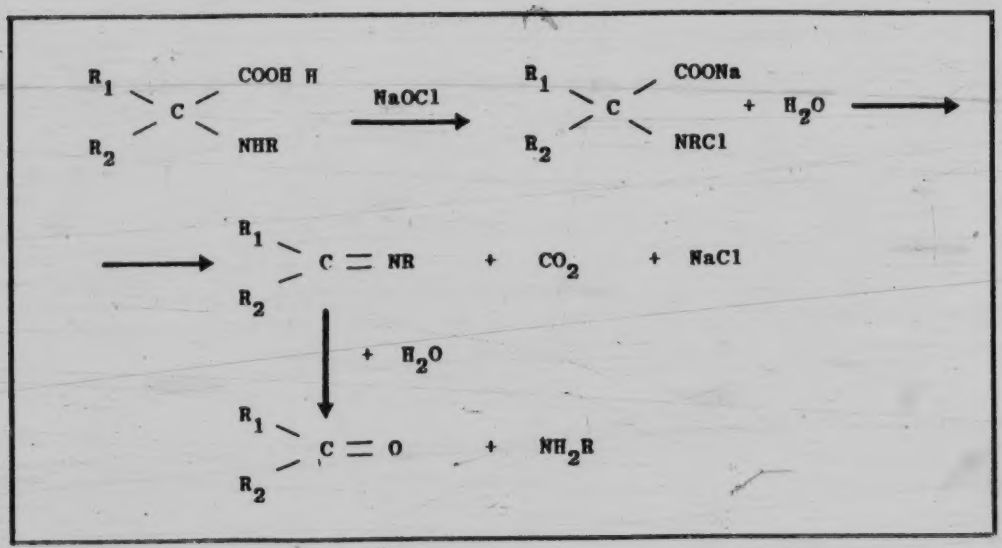


Figure 2.6 Chlorination pathways of amino acids.
(adapted from Stanbro and Smith, 1979)

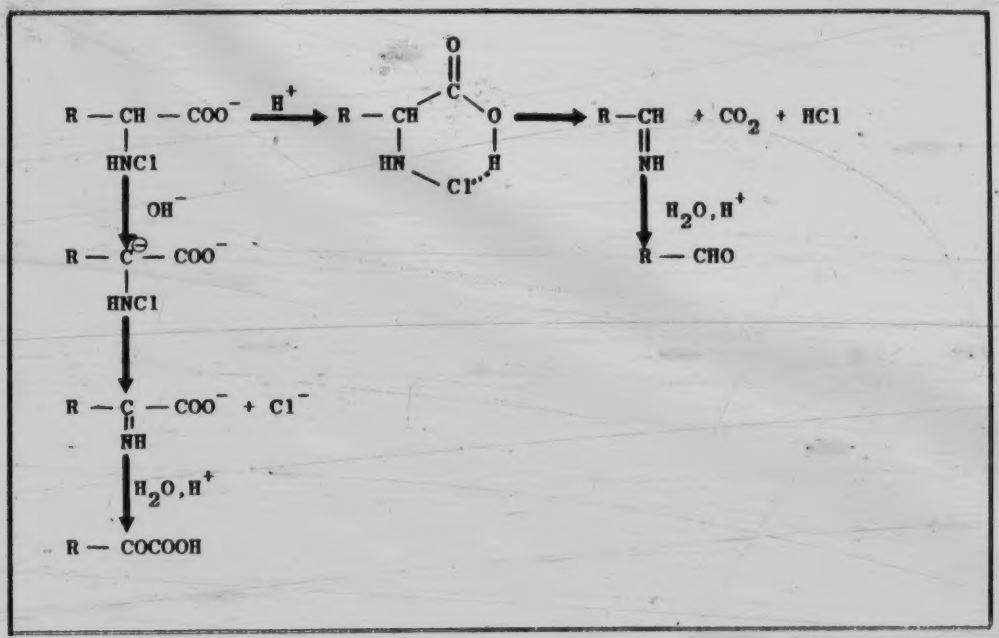


Figure 2.7 Effect of pH on product formation from chlorination of amino acids.

(adapted from Fox and Bullock, 1951)

Inglos et al. (1953), investigating chlorination of alanine, glycylglycine, glycylglycylglycine and tyrosine, obtained corresponding α-keto carboxylic acids. Stanbro and Smith (1979) reported the formation of acetaldehyde and pyruvic acid as the oxidation products of alanine, depending on the pH. The hypohalite-induced oxidative decarboxylation of various primary and secondary α-amino acids investigated by van Tamelen et al. (1968), led to the formation of aldehydes or α-keto carboxylic acids, tertiary α-amino acids, following N-chloroamino acid formation.

Several amino acids were shown to yield chlorinated products. Reactions of tyrosine with HOCl were reported to give a number of products including mono and dichlorohydroxybenzylnitriles and dichlorohydroxybenzaldehyde (Pereira et al., 1973). Substitution of Cl in the aromatic ring of tyrosine was also reported by Stanbro and Smith (1979) and by Shimizu (1973a, 1973b). The latter reported that, depending on the molar ratio of chlorine to the amino acids, three products were formed (Figure 2.9).

The reaction of chlorine and cystine was proven to be very rapid and, depending on the excess of chlorine, yielded either sulfonyl-keto-acid or chloroderivatives of cytosine as a cytosine-hypochlorous acid reaction products. One equivalent of HOCl gave 4-N-chloro derivatives. Two or three equivalents of HOCl produced additionally di- and trichlorotyrosine, respectively. Five equivalents of HOCl yielded unstable tri- and tetrachloro derivatives.

The reaction of histidine with NaOCl gave cyanomethyl-limidazole (Shimizu, 1973a), while histidine-HOCl reactions investigated by Murphy et al. (1975) produced nitrile. Chlorination of the aromatic ring of phenylalanine has been reported only by Yoshioka et al. (1980), who obtained 2,2 dichlorophenylacetaldehyde as the reaction product of phenylalanine. Phenylalanine chlorination studies, performed by Le Cloirec and Martin (1985), showed the formation of phenylacetaldehyde.

Methionine was reported to give dichloroacetonitrile ($CHCl_2CN$) (Horth et al., 1987). Dichloroacetonitrile was also detected by Trehy et al. (1986) while investigating chlorination by-products of aspartic

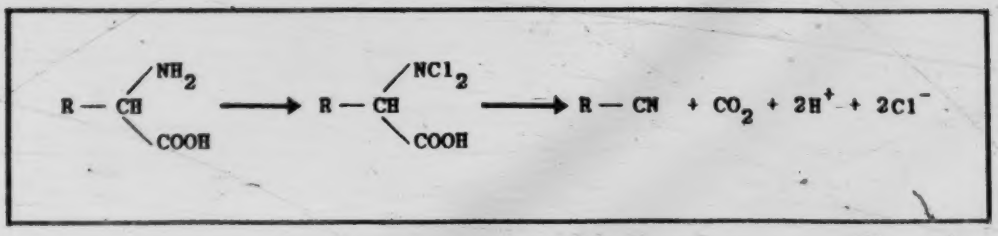


Figure 2.8 Decomposition of N, N dichloro-alpha-amino acid as intermediate step toward nitrile formation.
(adapted from Dakin, 1916)

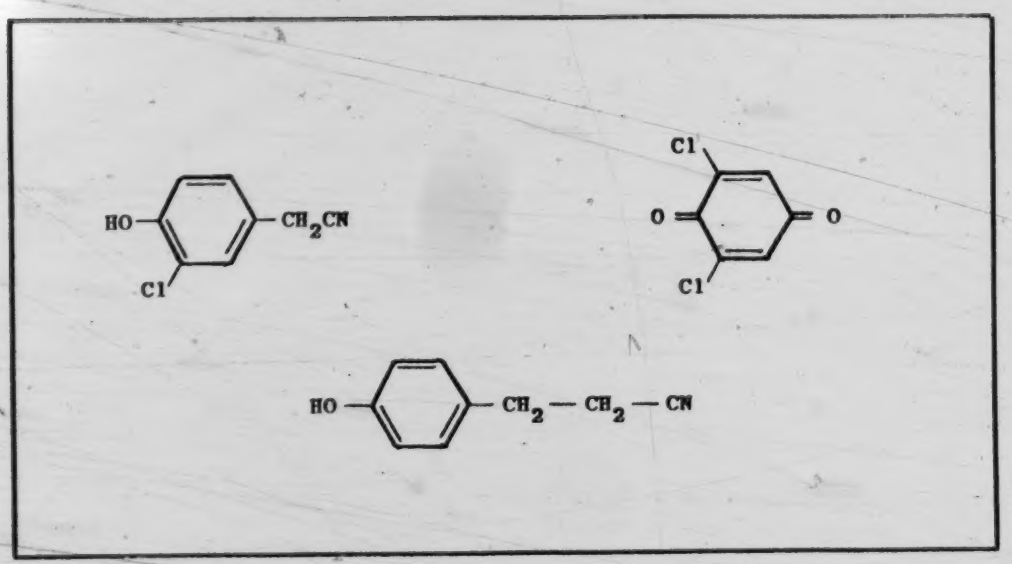


Figure 2.9 Chlorine/tyrosine reaction products.
(adapted from Shimizu, 1973a)

acid, tyrosine and tryptophan. The other major product was found to be chloral (CCl_3CHO).

Morris and Baum (1978) have identified proline and hydroxyproline as potential precursors for chloroform formation during chlorination.

While some authors did not report the formation of nitriles at all, and some claimed to obtain only nitriles as amino acids oxidation products, others have proved that both aldehydes and nitriles are formed.

Although both aldehydes and nitriles are formed during amino acid chlorination, their relative amounts are the function of: pH, molar ratio of amino acid to the oxidant and the presence of catalyst such as ferric ion (Yoshioka et al., 1980).

Investigating the reactions of HOCl with alanine at the pH of 5, 7 and 9 at 18°C, Le Cloirec and Martin (1985) conclude that there is a direct relationship between the nitrile and aldehyde concentrations and the pH. At higher pH the ratio of aldehyde to nitrile increases. This influence of pH on aldehyde/nitrile formation has been confirmed by others (Dakin, 1916, Pereira et al., 1973, Horth et al., 1987, Yoshioka et al., 1980, Kantouch and Abdel-Fatah, 1971).

Wright (1926), Norman (1936) and Yoshioka et al. (1980) who all showed the importance of pH on nitrile formation stress that at the neutral pH the reactions are more rapid and complete.

The relationship of nitrile and aldehyde formation influenced by pH can be summarized by schematic diagram proposed by Friedman and Morgulis (1936) (Figure 2.10). Although it shows the oxidation of amino acids by sodium hypobromite, it exactly follows the reaction pathways of chlorine derivatives with amino acids which are most often agreed upon in the literature.

Another important parameter influencing the product formation is the ratio of chlorine to amino acids (Dakin, 1916, van Tamelen et al., 1968, Shimizu, 1973b, Trehy et al., 1986, Yoshioka et al., 1980, Murphy et al., 1975). Yoshioka et al. (1980) for example, investigating chlorination of phenylalanine, showed that if HOCl/phenylalanine ratio is 1/1 or 1/2, mainly phenylacetaldehyde is formed, whereas if the ratio is 2/1, phenylacetonitrile is a major

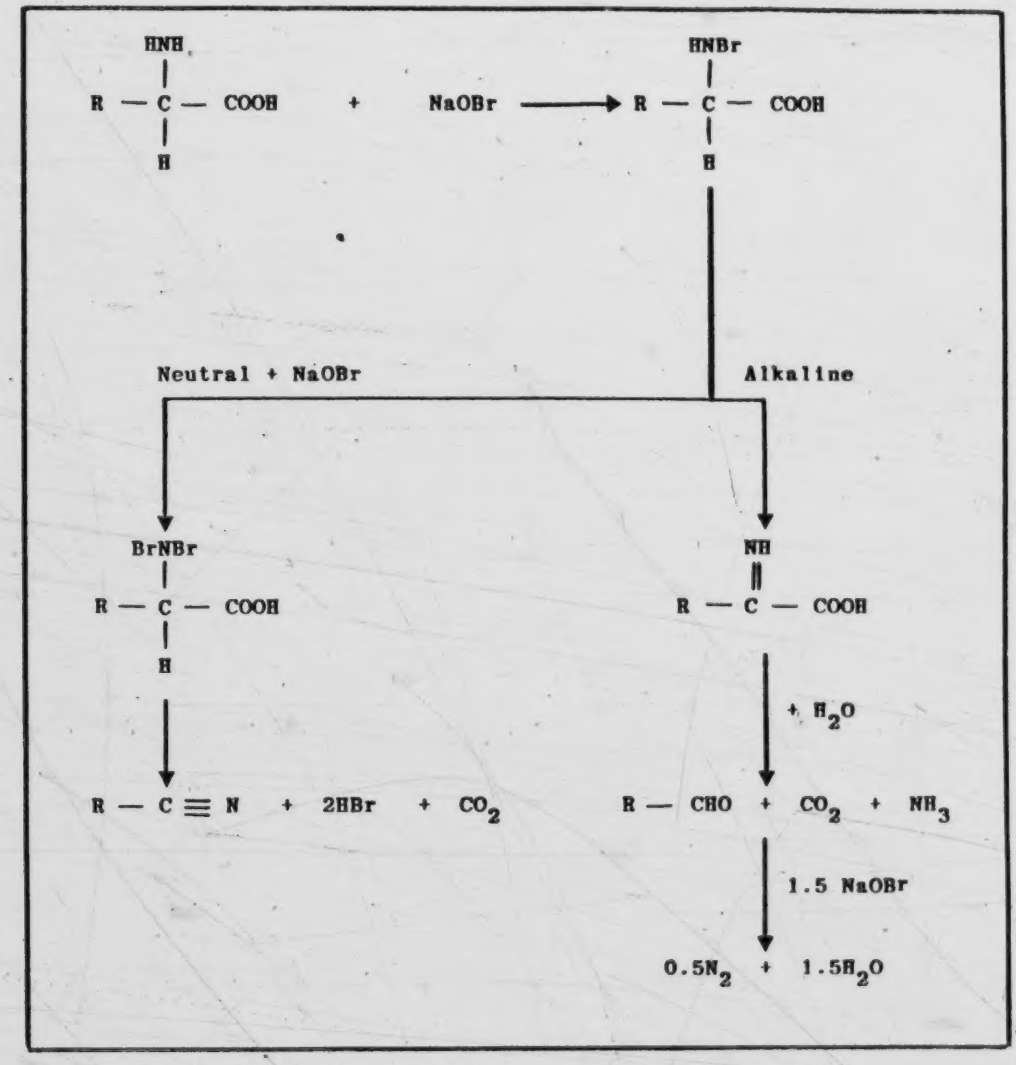


Figure 2.10 Oxidation of amino acids by sodium bromite (adapted from Friedman and Morgulis, 1936)

product. If the ratio is 5/1, both compounds are present in addition to benzaldehyde and 2,2 dichlorophenylacetaldehyde.

One work negating the influence of the oxidant/amino acid ratio is that of Shimizu (1973a), in which he claimed that free amino acids reacting with chlorine were actually converted to nitrile derivatives regardless of the relative amount of chlorine present.

The catalytic effect of ferric ion on amino acid-hypochlorite reactions is presented by Yoshioka et al. (1980). They show that in the reaction of phenylalanine with HOCl, ferric ion greatly enhanced the reaction rate for formation of both phenylacetaldehyde and phenylacetonitrile at the pH of 5.2. At that pH, Fe^{2+} also markedly favoured the phenylacetonitrile over phenylacetaldehyde formation. At the pH of 7.2, the significant catalytic effect of Fe^{2+} was observed only for higher HOCl/amino acid ratios. No catalytic effect of manganese was observed for manganese in these studies.

No reports of chlorination of valine, or isoleucine with the subsequent formation of corresponding aldehydes upon chlorination were found in the literature surveyed. The formation of phenylacetaldehyde as a chlorination product of phenylalanine has however been documented by Pereira et al. (1973), Murphy et al. (1974), Yoshioka et al. (1980), Le Cloirec and Martin (1985) and Horth et al. (1987). Isovaleraldehyde as a chlorination product of leucine was reported by Dakin (1916).

2.5.2.2 Reactions of amino acids with chlorine dioxide.

Review of the available literature demonstrates that relatively little work on reactions of chlorine dioxide with amino acids in aqueous solution has been done to date.

However, since chlorine dioxide has been used to improve the baking quality of flour, the reactions of chlorine dioxide with amino acids of wheat proteins have been well documented in the food science literature. Some of the findings from the research in this field, as implied but not scientifically verified by several authors, could apply to reactions of chlorine dioxide with amino acids in

aqueous solution. A summary of those findings and implications follows.

The early works investigating the reactions of chlorine dioxide with amino acids of wheat proteins include the works done by Moran et al. (1953) and Meredith et al. (1956). According to Moran et al. (1953), both pure chlorine dioxide and chlorine dioxide containing approximately 30% chlorine were used to treat flour. They found that chlorine dioxide reacted with methionine, cysteine and tyrosine. If pure chlorine dioxide was used, oxidation reactions predominated with the formation of cysteic acid and oxides of methionine (methionine sulfone and methionine sulfoxide). If free chlorine was also present the halogenation occurred. Under those circumstances, the presence of monochlorotyrosine and, at higher levels of chlorine, dichlorotyrosine was usually noted.

Meredith et al. (1956) treated wheat flour with high levels of chlorine dioxide. Although no protein or amino acid chloroderivatives were detected, the amounts of tryptophan and cystine were reduced to about 92% and 75% of their original levels respectively.

Reactions of chlorine dioxide with wheat flour, investigated by Fujii and Udita (1959), revealed that tyrosine and tryptophan reacted with chlorine dioxide giving colour substances. Under experimental conditions of pH 5.2 (wheat protein solution was dissolved in 0.005N AcOH), tryptophan was oxidized to complex mixtures containing indoxyl, isatine and indigo red besides unidentified chlorinated products, probably polymerized (Figure 2.11). The tyrosine and chlorine dioxide reaction produced dopaquinone and dopachrome (Figure 2.12), (Masschelein, 1979).

A colour producing reaction of chlorine dioxide with tyrosine was investigated by Hodgen and Inglos (1954) to develop a colorimetric method for determination of chlorine dioxide in water. They suggest that an excessive amount of chlorine dioxide causes the aromatic ring of tyrosine to cleave subsequently leading to alanine production.

Inglos et al. (1953) reported that chlorine dioxide did not react with alanine and glycylglycylglycine. Their study on reactions of selected amino acids with chlorine, chloramines and chlorine dioxide,

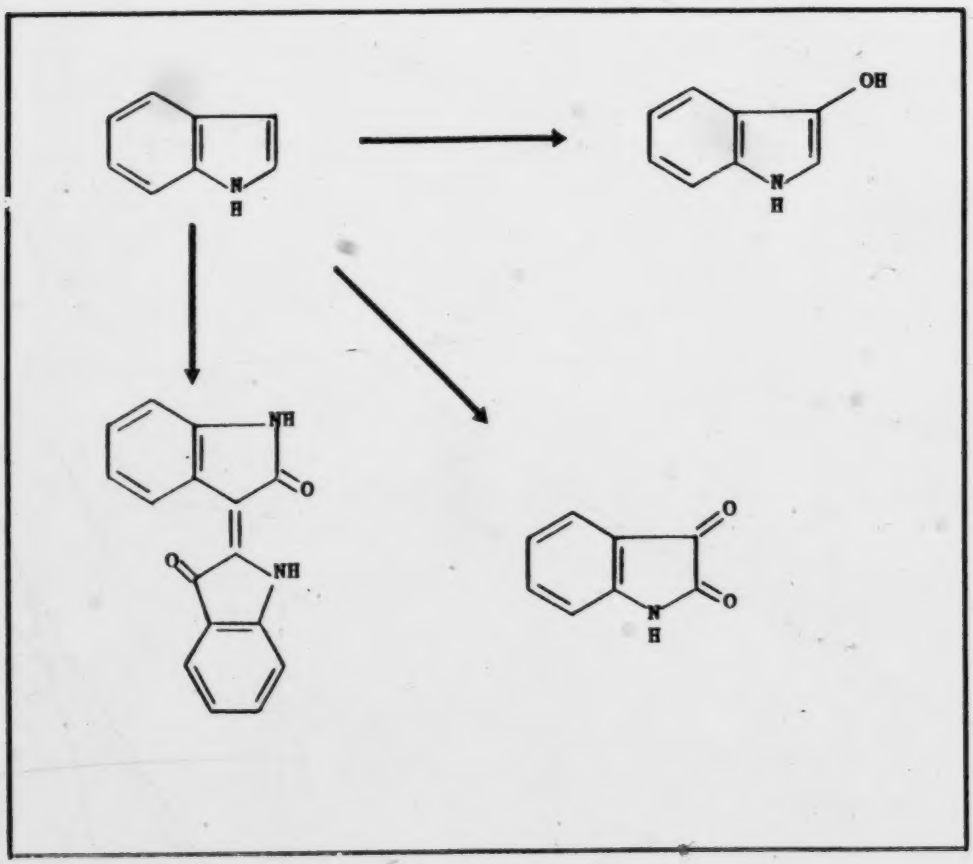


Figure 2.11 Chlorine dioxide/tryptophan reaction products.
(adapted from Masschelein, 1979)

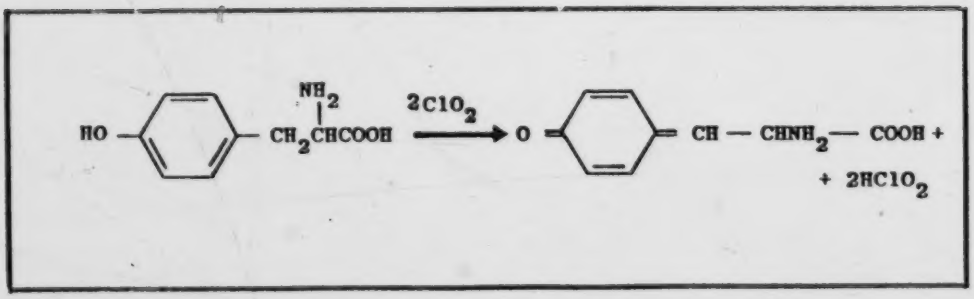


Figure 2.12 Chlorine dioxide/tyrosine reaction products.
(adapted from Masschelein, 1979)

performed to evaluate the importance of various mechanisms involved in bacterial death, implies that chlorine dioxide does not attack the amino radicals in amino acids. It, however, oxidizes the aromatic ring in case of tyrosine. The reaction product of cytosine and chlorine dioxide was reported to be sulfonyl amino acid ($HSO_3CH_2CHNH_2COOH$).

Kennaugh (1957) studied the reactions of amino acids with diaphanol (50% acetic acid saturated with ClO_2). Performing tests on individual amino acids he found that after 18 hours phenylalanine had been partially broken down leading to formation of alanine. After treatment for 24 hours with diaphanol, both ortho- and paratyrosine were absent, and proline was also partially eliminated. When a mixture of tryptophan, orthotyrosine, paratyrosine, proline, threonine, methionine, valine and glycine was placed in diaphanol the aromatic acids had disappeared after 18 hours and proline was reduced in amount. All the acids had been destroyed after 5 days.

Based on these results, Rav-Acha (1984), in his article reviewing reactions of chlorine dioxide with aquatic organic materials, concluded that amino acids not containing specific reactive groups (eg. glycine, alanine, phenylalanine, serine and leucine) did not react with chlorine dioxide under water treatment conditions. Katz (1980) relying upon the same source, concluded that glycine, alanine and phenylalanine were stable to oxidation by chlorine dioxide.

Often cited is work done by Schmidt and Bransdort (1922) who, as reported by Masschelein (1979), found that among the amino acids the least reactive with chlorine dioxide are glycine, leucine, serine, alanine, phenylalanine, valine, hydroxyproline, aspartic and glutamic acids.

Investigating the possible action of chlorine dioxide on amino acids of proteins within the bacterial cell, Benarde et al. (1965) treated each of six amino acids (histidine, asparagine, phenylalanine, arginine, proline and leucine) with chlorine dioxide for a period of 30 minutes. From that set of experiments, based on the determination of unreacted amino acids, they concluded that chlorine dioxide did not

react with amino acids sufficiently to alter their original characteristic structures.

The reactivity of amino acids with chlorine dioxide was investigated by Noss at al. (1981) by adding equimolar concentrations ($2.5x10^{-4}$) of chlorine dioxide to each of the 22 amino acids at pH 7.0. Loss of at least 10% of the starting chlorine dioxide concentration within 1 hour was judged to indicate reactivity. From the findings of this research it appears that under the experimental conditions employed only hydroxyproline, proline, histidine, cysteine, cystine, tyrosine and tryptophan are susceptible to chlorine dioxide oxidation.

Palin (1967) assumed that glycine did not react with chlorine dioxide and used it as one of the reactants in his method for determining chlorine concentration in a chlorine dioxide solution.

In disagreement with the above reports is the work done by Taymaz et al. (1979) in which reactions of glycine and chlorine dioxide in aqueous solution at 70°C resulted in the formation of formaldehyde. When phenylalanine was treated with chlorine dioxide (under the same conditions) the following reaction products were identified: phenylacetic acid, phenylacetaldehyde, benzoic acid, benzaldehyde and mandelic acid. However, the results should be regarded with caution noting the importance of experimental conditions. The reactions of glycine and phenylalanine carried out at 0°C, 23°C and 55°C show that the reaction rate is temperature dependent and that the highest reactivity occurs at 55°C. In case of glycine as well as phenylalanine it appears to be a rapid initial reaction followed by the slower secondary reaction. When experiments with phenylalanine were carried out at room temperature with two molar equivalents of chlorine dioxide for 2 days, only 4% of the initial phenylalanine content reacted suggesting a very slow reaction rate.

Rice and Gomez-Taylor (1986) also report that chlorine dioxide will convert glycine to formaldehyde while phenylalanine will produce traces of benzylic acid, benzoic acid, mandelic acid and phenylacetaldehyde.

A thorough examination of the foregoing findings leads to the conclusion that the reactivity of the amino acids of interest; valine, isoleucine, leucine and phenylalanine with chlorine dioxide under water treatment conditions is unclear and that definitive studies on this subject are yet to be done.

Some researchers (Schmidt and Bransdort, 1922, Benarde et al., 1965, Rav-Acha, 1984, Noss et al., 1981) claim that these simple amino acids are stable to chlorine dioxide oxidation.

Two reports (Hodgen and Inglos, 1954, Kennaugh, 1957) show that chlorine dioxide reacts with phenylalanine producing alanine. This in turn suggests that the aromatic ring of phenylalanine is cleaved in the course of reaction.

Traces of phenylacetaldehyde have been reported as a product of phenylalanine and chlorine dioxide reaction by Taymaz et al. (1979) and Rice and Gomez-Taylor (1986). However, these reports are not clear about the possibility of chlorine being present in the chlorine dioxide solution and being responsible for the aldehyde yield observed.

2.5.2.3 Reactions of amino acids with monochloramine.

The reactions of monochloramine (NH_2Cl) with amino acids have been mainly evaluated with respect to bacterial deactivation (i.e. in order to determine possible target sites for the disinfectant within proteins). Only relatively limited literature discussing these processes has been found.

One of the earliest studies on the reactions with amino acids was conducted by Dakin (1916). He found that chloramine-T (N-chloro-p-toluenesulfonamido sodium) reacted with these compounds to form mono- and dichloro- substitution products depending on the concentration of chlorine and the number of hydrogen groups attached to the nitrogen. Dakin (1916) also indicated that there was no difference, other than rate, between the reactions of amino acids with hypochlorite and with chloramines. When one equivalent of the chloramine was used, α-amino acids were converted to their corresponding aldehydes while if two equivalents of reagents were

used the corresponding nitriles resulted. Guiteras and Schmelkes (1934) showed through chloramine-T studies that the sulfur containing amino acids reacted most readily. Jacangelo and Olivieri (1984a) also show that the most reactive amino acids were those containing sulfur residues (cysteine, cystine and methionine) and a ring containing tryptophan. All consumed 100% monochloramine in less than 2 minutes. The least reactive amino acid appears to be glycine. Jacangelo and Olivieri (1984b) present a list of consumption of monochloramine by amino acids at pH 7.0 and 23°C after 2 and 180 min. reaction times. This list is presented in Table 2.4.

Inglos et al. (1953) studied chloramine reactions with alanine, cytosine and tyrosine. They report that alanine and monochloramine form a mixture of the organic and inorganic monochloramines. When cysteine was reacted, the oxidation of the sulfhydryl was carried to the disulfide state, forming cystine. No further oxidation of the sulfur group occurred. Tyrosine did not react appreciably with monochloramine.

Isaac and Morris (1980) investigated chlorination of nitrogenous substrates in wastewater effluents including glycine, alanine and serine. By observing the kinetics of reactions, the authors concluded that a transfer of Cl^+ from monochloramine to organic nitrogen occurred. As a result, chloroamino acids were formed. They also showed two possible mechanisms for the chlorine transfer from NH_2Cl to organic nitrogen. One is hydrolysis (monochloramine is hydrolysed to hypochlorous acid which subsequently oxidizes a N-compound):

$$NH_2Cl + H_2O \text{ ------> } NH_3 + HOCl$$

$$\text{N-compound} + HOCl \text{ ------> } \text{N-chloro compound} + NH_3$$

The second mechanism is a direct transfer:

$$NH_2Cl + \text{N-compound} \text{ ------> } \text{N-chloro compound} + NH_3$$

Table 2.4. Precent consumption of monochloramine by amino acids.

amino acid	percent NH_2Cl consumed after 2 min	after 180 min
alanine	0	86
cystine	100	100
phenylalanine	2	96
tyrosine	10	100
cysteine	100	100
isoleucine	3	99
serine	6	98
glycine	0	6
threonine	10	82
histidine	11	100
aspartic acid	8	100
glutamic acid	0	99
tryptophan	100	100
valine	2	93
lysine	3	100
glutamine	3	97
arginine	8	98
asparagine	2	100
proline	2	90
methionine	100	100
leucine	13	95

Both reactions depend on the concentration of chloramines and N-compounds. In effluents and receiving waters (substrate concentration 10^{-4} M) direct transfer will occur primarily. In fresh waters (substrate concentration 10^{-5} M), both mechanisms will be important in the chlorine transfer. Subsequent studies fully confirmed these findings (Isaac and Morris, 1983). Moreover, the reactions of monochloramine with amino acids tested were observed to be second order. The reaction rate constants varied little over a pH range from 6.5 to 8.5.

The earlier work by Morgerum et al. (1978), concerned with the kinetics of chlorine transfer to N-compounds, show that the direct transfer mechanism is the main reaction pathway ($k=1.5\ M^{-1}s^{-1}$) hydrolysis being practically negligible ($k=1.9 \times 10^{-5}\ M^{-1}s^{-1}$). This has been confirmed by a more detailed studies of the kinetics of chlorine transfer from chloramine to amines, amino acids and peptides conducted by Snyder and Margerum (1982) who also proposed that protonated chloramine (NH_3Cl^+) was a very reactive chlorination agent. The reaction pathway is as follows:

$$NH_2Cl + H^+ \quad <======> \quad NH_3Cl^+$$

$$NH_3Cl^+ + R\text{-}NH_2 \quad ------> \quad R\text{-}NHCl + NH_4^+$$

The rate of chlorine transfer to amino acids is pH dependent and increases in acid solution. This study did not identify the reaction products other than organic monochloramines. However, oxidative decarboxylation of N-chloro-L-threonine and the decay reaction of organic monochloramine were observed.

Patton et al. (1972) provide an interesting insight to the possibilities of the decomposition of N-chloroamines formed. During this study, phenylalanine was treated with the organic monochloramine, 4-N-chlorocytosine, for one hour in water. Ether extraction followed by the GS-MS analysis identified phenylacetaldehyde as a reaction product. The decomposition of chloroamino acids as reaction products of monochloramination was also considered by Rice and Gomez-Taylor (1986). They draw

attention to the rapid decomposition of N,N-dichloro-α-amino acids as a source of nitrile in chloraminated waters.

2.5.2.4 Reactions of amino acids with ozone.

The literature presents very little information on the reactions of ozone with amino acids and their reaction products. The research conducted to date was mainly concerned with reaction kinetics.

Pryor et al. (1984) studied absolute rates of reaction of several amino acids with ozone in aqueous solution at different pH values. The reactivity was measured by determining the amounts of amino acids consumed in the reaction. The products were not identified. The reactions were found to be pH dependent. At low pH the reported order of reactivity was: tryptophan > methionine > tyrosine > histidine > cystine > phenylalanine >> all others. At high pH, the order of reactivity changed to : cysteine > tryptophan > methionine > tyrosine > histidine > cystine > phenylalanine >> all others. The foregoing findings were consistent with those of Mudd et al. (1964) and Hoigne and Bader (1983). Pryor et al. (1984) further indicate that phenylalanine reacts faster than the aliphatic amino acids, presumably due to the reactivity of the aromatic ring. Glycine reacts about four times as fast as an average aliphatic amino acid. Hoigne and Bader (1983) also found glycine to be more reactive toward ozone than was alanine. Spanggord and McClurg (1976) report that 22% of glycine reacted when it was treated with ozone for 130 min. Rice and Gomez-Taylor (1986) conclude; "The amino acid alanine is decomposed readily by ozonation liberating all of the organic-nitrogen as both ammonium and nitrate ions. Phenylalanine is less readily decomposed by ozone, and 30% to 75% of the organic nitrogen remains combined in the organic oxidation products."

Generally, the reactivity of amino acids towards ozone is attributed to the α-amino group (Katz, 1980, Pryor et al., 1984, Spanggord and McClurg 1976). However, in amino acids possessing a side-chain group (i.e. cysteine, tryptophan, methionine), this active group is a site of reaction rather than the α-amino group.

3. EXPERIMENTAL METHODS AND CONDITIONS

The experimental technique used for this study involved analysis of four aldehydes; isobutyraldehyde, 2-methyl butyraldehyde, isovaleraldehyde and phenylacetaldehyde generated from reactions of corresponding amino acids with four oxidants; chlorine, monochloramine, chlorine dioxide and ozone. Aldehydes produced from valine, leucine and isoleucine were analyzed by purge and trap with subsequent thermal desorption and cryogenic/temperature programmed gas chromatography according to the method developed for this study. Phenylacetaldehyde, the product of phenylalanine, was analyzed chromatographically by a direct aqueous sample injection method.

3.1 Sample preparation and handling.

All the glassware used for experiments was washed in a dishwasher with Heikol detergent and phosphoric acid, rinsed with distilled water warmed up to 85°C and then oven-dried at 65°C.

The procedure for aldehyde generation was the same for all experiments. Standard amino acid solutions were prepared freshly prior to each set of experiments using analytical grade chemicals (Aldrich). Water was purified with a Milli-Q™ apparatus. To prepare amino acid samples, a desired amount of amino acid stock solution was pipetted to 1 liter volumetric flasks serving as reaction flasks. Samples were buffered to a well defined pH by adding 20 mL of inorganic buffer (Fisher Scientific) and then treated with an appropriate amount of oxidant to obtain a predetermined oxidant/amino acid molar ratio. The composition of buffers is as follows:

- pH 6 potassium phosphate monobasic-disodium phosphate
- pH 7 potassium phosphate monobasic-sodium hydroxide
- pH 8 potassium phosphate monobasic-sodium hydroxide

- pH 9 boric acid-potassium chloride-sodium hydroxide
- pH 10 potassium carbonate-potassium borate-potassium hydroxide

The reactions were carried out in darkness. The reactions were allowed to proceed at the temperature of 4°C by placing the samples in an incubator, or at room temperature controlled to 20°C. At the end of a desired reaction time, an excess amount of sodium thiosulfate ($Na_2S_2O_3$) was added to eliminate any residual oxidant. Toluene internal standard was added to samples analyzed for isobutyraldehyde, 2-methyl butyraldehyde and isovaleraldehyde, and the solution was filled up with water to 1 litre volume. No internal standard was added to samples analyzed for phenylacetaldehyde.

3.2 Analytical methods.

Analytical methods employed wet and instrumental chemistry. Wet chemistry included titrimetric analysis for determining concentrations of oxidants in solutions used for experiments. Instrumental chemistry included gas chromatography analysis.

3.2.1 Determination of oxidant concentration.

Concentrations of oxidants were determined before use. Chlorine stock solution was prepared from the purified grade 4 to 6% sodium hypochlorite (NaOCl) solution (Fisher Scientific) and its concentration was determined by the iodometric method (Standard Methods, 1985).

Monochloramine solution was prepared by reacting ammonium chloride (NH_4Cl) with aqueous chlorine at a 3 to 1 molar ratio and pH 8.4 according to the method described by Gordon et al. (1987). Monochloramine solution was prepared before use and its concentration was determined by the amperometric method (Standard Methods, 1985).

Chlorine dioxide solution containing approximately 4.6% chlorine was obtained from the City of Edmonton Rossdale Water Treatment Plant. Chlorine dioxide and its chlorine content concentrations were measured by the amperometric method of Aieta et al. (1984) by titrating with standardized 0.1 N sodium thiosulfate.

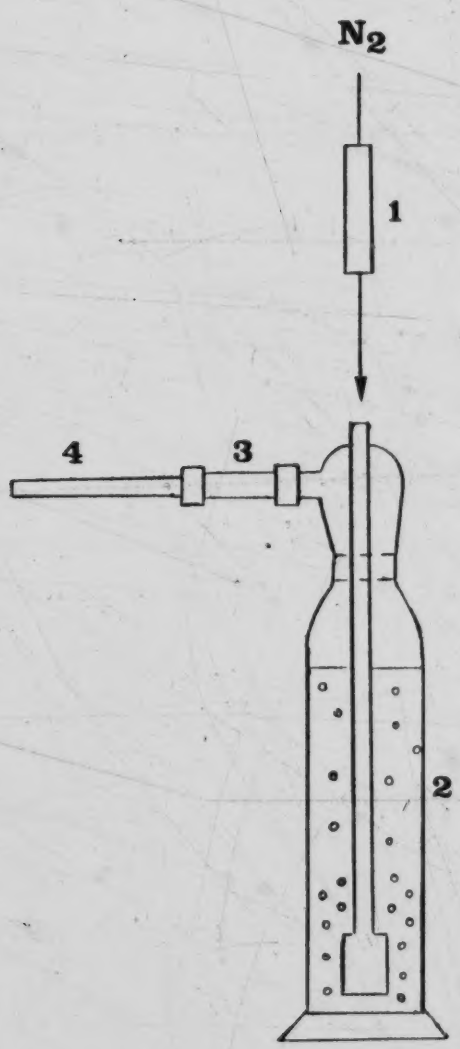
The solution of ozone was obtained from a colleague responcible for ozone genaration. It was prepared by bubbling extra dry ozonized oxygen through 400 mL Milli-Q purified water in a 500 mL gas washing flask for approximately 40 min. Ozone concentration varied between 9 and 18 mg O_3/L and was determined using the potassium iodide method of Birdsall et al. (1952). This method required transferring accurately measured volume of ozone stock solution to 2% potassium iodide solution followed by titrating with standardized 0.01 sodium thiosulfate at pH 2. To measure the exact amount of ozone added to samples, each set of samples was accompanied by 200 mL of potassium iodide to which 50 mL of ozone rich solution was added and titrated immediately.

3.2.2 Analysis of isobutyraldehyde, isovaleraldehyde and 2-methyl butyraldehyde.

The aliphatic aldehydes; isobutyraldehyde, 2-methyl butyraldehyde and isovaleraldehyde were subjected to direct sorption onto the sorbent (Tenax GC) which provided a means of concentrating the sample components. Thermal desorption of the trapped aldehydes onto the glass capillary chromatographic column and gas chromatographic analysis provided both qualitative and quantitative data.

3.2.2.1 Purge and trap method.

The purging apparatus used in experiments consisted of glass gas washing bottles and teflon, glass and stainless steel tubing. The schematic diagram of the apparatus is presented in Figure 3.1



1 gas purifier

2 gas washing bottle

3 teflon tube with Na_2SO_4

4 adsorbent tube containing Tenax

Figure 3.1 Schematic diagram of the purging apparatus.

125 mL of a reacted solution with 13.9 µg/L toluene internal standard was purged with nitrogen gas at a flow rate of 30 mL/min ± 3 for 60 min through 110 mm x 2 mm ID borosilicate glass tubes. Each tube contained 65 mg Tenax-TA™ adsorbent. Subsequently, the samples (adsorbent tubes) were thermally desorbed for 2 min at 200°C directly into a capillary column using Supelco Envirochem Thermal Tube Desorber Model 850.

3.2.2.2 Instrumental method.

The GC analysis was performed within a few hours following sorption to prevent aldehyde loss from Tenax. The chromatographic conditions were as follows. A capillary column (30 m x 0.32 mm SPB-5) was held for 1 min at -30°C. The GC oven was programmed to reach the temperature of 250°C at a rate of 10°C/min. The analyses were performed on the GC model HP 5890A with a flame ionization detector (FID) held at 300°C and helium as a carrier gas. Running the full temperature program was not necessary since very few higher molecular weight compounds existed in the experimental water samples. Consequently, the runs were usually terminated after toluene, the last peak of interest, had been eluted from the column. Peak areas were measured with a HP 3392A integrator.

Quantitation of the results was achieved by means of calibration curves of aldehyde concentration vs. aldehyde to internal standard intensity ratio. Calibration curves were obtained by using 125 mL aliquots of solution containing 0.5 to 100 µg/L aldehydes and 13.9 µg/L toluene internal standard. Aldehyde standard solutions were prepared by adding a carefully measured volume of aldehyde stock solution (Aldrich) and toluene stock solution (Baker Chemicals) to known volume of deionized water in a glass bottle containing no head space. The solutions were left for 24 hours and were frequently stirred to assure that the chemicals were completely dissolved in water. The calibration curves for each aldehyde are presented in Appendix B.1. This method had a linear range of 0.5

µg/L to 120 µg/L with an average precision of 5% as relative standard deviation.

3.2.3 Analysis of phenylacetaldehyde.

Determination of phenylacetaldehyde was carried out by injecting 3 µL of the aqueous sample onto a 2 m x 2 mm glass column packed with 10% Fluorad FC-431 on Chromosorb W (80/100 mesh) with helium as a carrier gas. The analyses were performed on a Varian 3300 gas chromatograph equipped with an FID. The injection port was operating at 200°C. The oven temperature was held at 110°C. The temperature of the detector was 300°C. The runs were terminated after the last peak of any impurity had been eluted, usually after 10 to15 min. Peak areas were measured with a Varian SP4290 integrator.

Quantitation of phenylacetaldehyde was achieved by means of the calibration curve of aldehyde concentration versus peak area counts which is presented in Appendix B.2. This method had a lenear range of 0.5 mg/L to 20 mg/L with an average precision of 8% as relative standard deviation.

3.3 Experimental conditions.

Conditions chosen for the experiments varied over the range commonly applied to water treatment. Factors studied included pH, reaction time, reaction temperature and oxidant/amino acid molar ratios. All the experiments were replicated to provide at least duplicate results.

3.3.1 Experiments with chlorine.

The preliminary tests included experiments designed to investigate the effects of reaction time, molar ratio and pH on the

highest aldehyde yield. Experiments were performed with all four amino acids at concentrations of 0.001 mM for valine, leucine and isoleucine and 0.1 mM for phenylalanine. The higher concentration of phenylalanine was necessary because of the poorer sensitivity of the analytical method for phenylacetaldehyde. The chlorine/amino acid molar ratio varied from 0.5 to 30.0. The reactions were conducted for 0.5 to 3 hours at the temperature of 20°C. The samples were buffered to pH 7 and 9.

To investigate effects of pH, reaction time and temperature, and their possible interactions, a 2^3 factorial experiment was performed. The amino acid chosen for this experiment was leucine and the molar ratio was 1.5 (which preliminary experiments suggested should give the maximum aldehyde yield).
The levels of 3 factors were set as follows;

Factor	levels	
	low	high
pH	7	9
time(h)	0.5	3.0
temp(°C)	4	20

To further investigate the effect of pH on an aldehyde yield from leucine/chlorine reaction, pH was varied from 6 to 10 at the molar ratio of 1.5.

3.3.2 Experiments with monochloramine.

For comparison, preliminary monochloramine/amino acid reaction experiments were performed with all four amino acids at concentrations and conditions the same as for the experiments with chlorine (0.001 mM for valine, leucine and isoleucine, and 0.1 mM for phenylalanine). The pH of the preliminary experiments was 7 and 9,

reaction time varied from 0.5 to 3 hours and the molar ratio ranged from 0.5 to 10.

More detailed experiments were performed with leucine. They included a 2^4 factorial experiment. The levels chosen for this experiment were as follows;

Factor	level	
	low	high
pH	7	10
molar ratio	5	10
reaction time (h)	6	24
reaction temperature (°C)	4	20

To investigate the effect of pH on monochloramine/leucine reactions the pH was varied from 6 to 10 and the reactions were allowed to proceed for 24 hours. Other experiments were performed for varying reaction times ranging from 6 to 24 hours at different molar ratios and pH values of 7, 8, and 10. Except for the factorial experiment investigating the effect of low temperature, the reaction temperature was always 20°C.

3.3.3 Experiments with chlorine dioxide.

Experiments involved treating leucine (0.001 mM) and phenylalanine (0.1 mM) with chlorine dioxide at the pH of 7 and 9. The reaction time was 2 hours, molar ratio varied from 0.5 to 79.0. The reactions were performed at 20°C.

3.3.4 Experiments with ozone.

Experiments involved reacting ozone with leucine and phenylalanine. The concentration of leucine in samples was increased to 0.01 mM and that of phenylalanine to 1.0 mM.

After the addition of ozonated water to samples, a molar ratio varied between 0.5 and 5 for leucine and between 0.5 and 0.9 for phenylalanine. Because of the low concentration of ozone stock solution and a high concentration of phenylalanine required to obtain detectable levels of phenylacetaldehyde, higher molar ratios were not achieved.

The mixtures of ozone and amino acids were maintained at room temperature for 5 to 60 min. The samples were buffered to pH 7 or 9.

4. RESULTS

4.1 Identification of aldehydes.

The aliphatic aldehydes were identified on the basis of their retention times relative to that of the internal standard, toluene. Phenylacetaldehyde was identified on the basis of its absolute retention time. Retention times of aldehyde peaks are listed in Table 4.1. Relative retention times rather than absolute retention times were used for compound identification because absolute retention times varied with the degree of packing of the Tenax-TA™ in the purge tubes and the resulting gas flow through them. A standard solution was analyzed with each set of samples (daily) to account for transient phenomena such as an instrument drift.

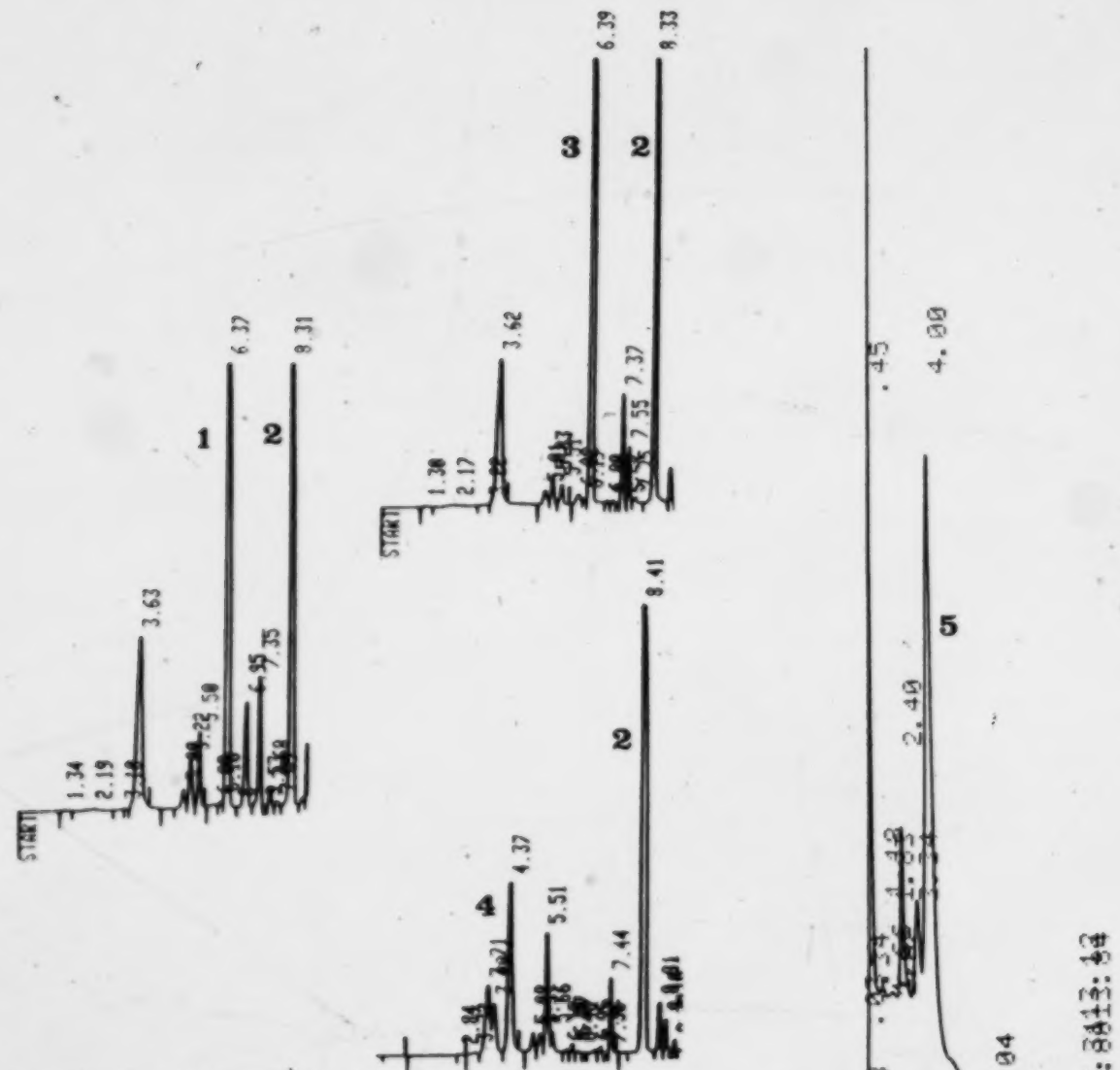
Typical chromatograms of the aldehydes as chlorination products of amino acids are presented in Figure 4.1. Resolved peaks are correspondingly labeled.

4.2 Production of aldehydes by reaction of oxidants with amino acids.

The major variables considered for the study were: oxidant/amino acid molar ratios, reaction time, pH and temperature. Experiments were performed with four different oxidants: chlorine, monochloramine, chlorine dioxide and ozone.

4.2.1 Experiments with chlorine.

4.2.1.1 Aldehyde formation. Chlorination of leucine, isoleucine, valine and phenylalanine show that these amino acids are converted to their corresponding aldehydes with very high yields.



1 2-methyl butyraldehyde

2 toluene

3 isovaleraldehyde

4 isobutyraldehyde

5 phenylacetaldehyde

Figure 4.1 Gas chromatograms of odorous aldehydes identified after chlorination of amino acids.

Table 4.1 Retention times of aldehydes.

compound	absolute retention time±std. dev.	relative retention * time±std. dev.
isobutyraldehyde	4.43±0.03	0.42±0.005
2-methyl butyraldehyde	6.30±0.03	0.69±0.008
isovaleraldehyde	6.55±0.03	0.72±0.008
phenylacetaldehyde	4.03±0.04	

* Toluene retention time 8.52±0.08

Calculations were based on 9 replicates.

4.2.1.2 Chlorine/amino acid molar ratio. Several experiments were performed to compare the yields of aldehydes at different chlorine dosage. The results obtained from these tests are plotted in Figures 4.2 and 4.3. These plots show the same pattern for all four aldehydes. Maximum yields were observed for the chlorine/amino acid molar ratio of 1.5. A comparison of results obtained from chlorination of aldehydes at molar ratios of 10 and 30 (Figure 4.3) shows that a higher dosage of chlorine did not decrease aldehyde yields.

4.2.1.3 Reaction time. The effect of reaction time on aldehyde formation is presented in Figures 4.4 and 4.5. Aldehyde yields increased progressively with time over the range of reaction time tested. The plots of % theoretical yields versus reaction time ranging from 15 min to 2 hours show that the yield of 2-methyl butyraldehyde increased from 10 to 60%, whereas the yield of isobutyraldehyde increased from 10 to 40% (Figure 4.5).

4.2.1.4 Reaction pH. The effect of a hydrogen ion concentration on aldehyde yields was tested over the pH ranging from 6 to 10. Aldehyde yields as exhibited by leucine conversion to isovaleraldehyde are not dependent on pH in the range from 6 to 8 (Figure 4.6). Chlorination of leucine, isoleucine and valine show that consistently higher yields of aldehydes were observed at pH 9 than at pH 7, although the differences were relatively small (Figure 4.7-4.9). This effect was not observed for phenylalanine formation (Figure 4.10). However, ANOVA (Appendix C.1) shows that population mean values at each pH are statistically different. Chlorination of leucine , isoleucine and valine shows that consistently higher yields of aldehydes were observed at pH 9 than at pH 7, although the differences were relatively small (Figure 4.7-4.9). This effect was not observed for phenylalanine formation (Figure 4.10).

4.2.1.5 Combination of factors. A factorial experiment was designed for chlorination of leucine. Results (Table 4.2) indicate that temperature had a major effect, reaction time had a lesser effect

while pH had only a marginal effect on isovaleraldehyde yield. No significant effects were observed for two and three factor interactions. Calculations of the effects are presented in Appendix C.2.

Maximum yields among reaction conditions tested for chlorine were observed at a chlorine/amino acid molar ratio of 1.5, reaction time of 2 hours, pH of 9 and temperature of 20°C. These yields were 66%, 74%, 85% and 87% for isobutyraldehyde, 2-methyl butyraldehyde, isobutyraldehyde and phenylacetaldehyde, respectively.

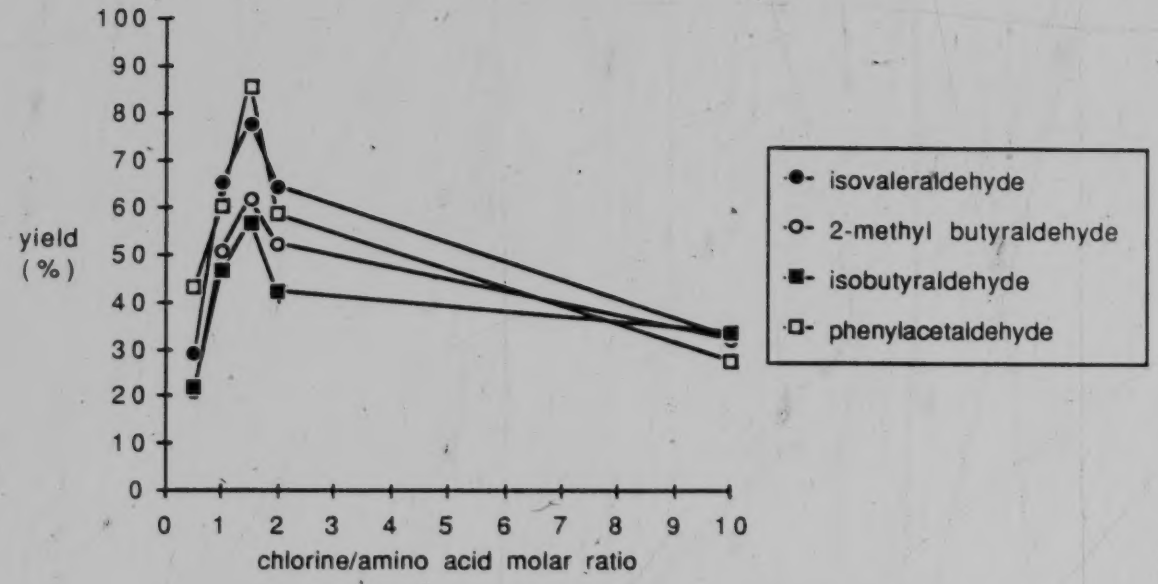


Figure 4.2 Formation of aldehydes at different chlorine/amino acid molar ratios. Reaction time = 2 h, pH = 7. Theoretical yield under experimental conditions: isovaleraldehyde = 86 µg/L, 2-methyl butyraldehyde = 86 µg/L, isobutyraldehyde = 72 µg/L, phenylacetaldehyde = 12 mg/L.

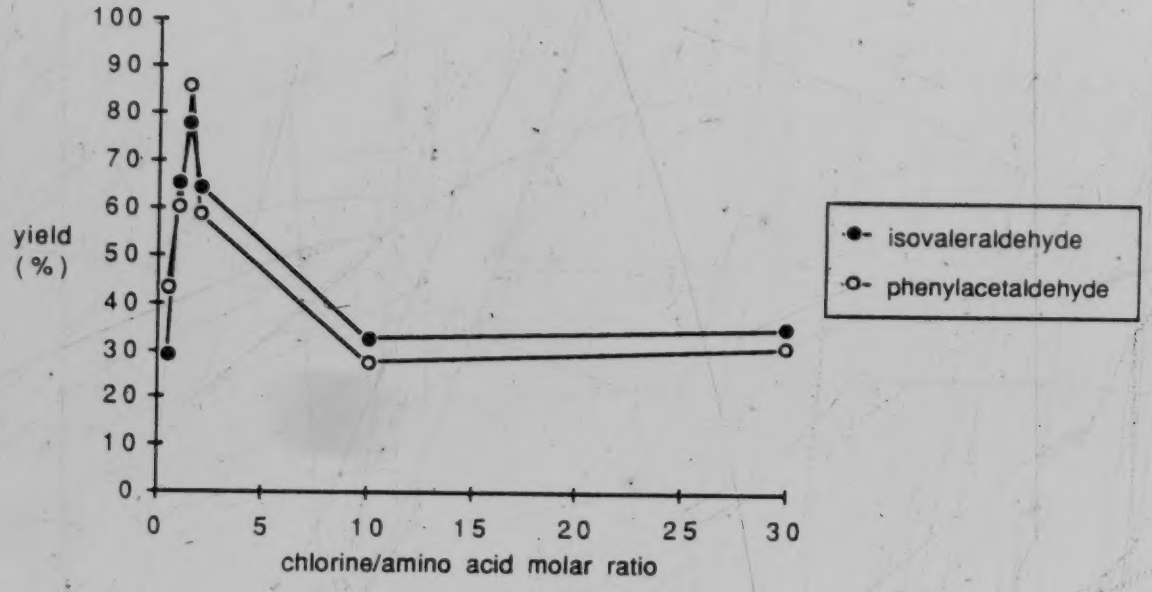


Figure 4.3 Effect of high dosages of chlorine on formation of aldehydes. Reaction time = 2 h, pH = 7. Theoretical yield under experimental conditions: isovaleraldehyde = 86 µg/L, phenylacetaldehyde = 12 mg/L.

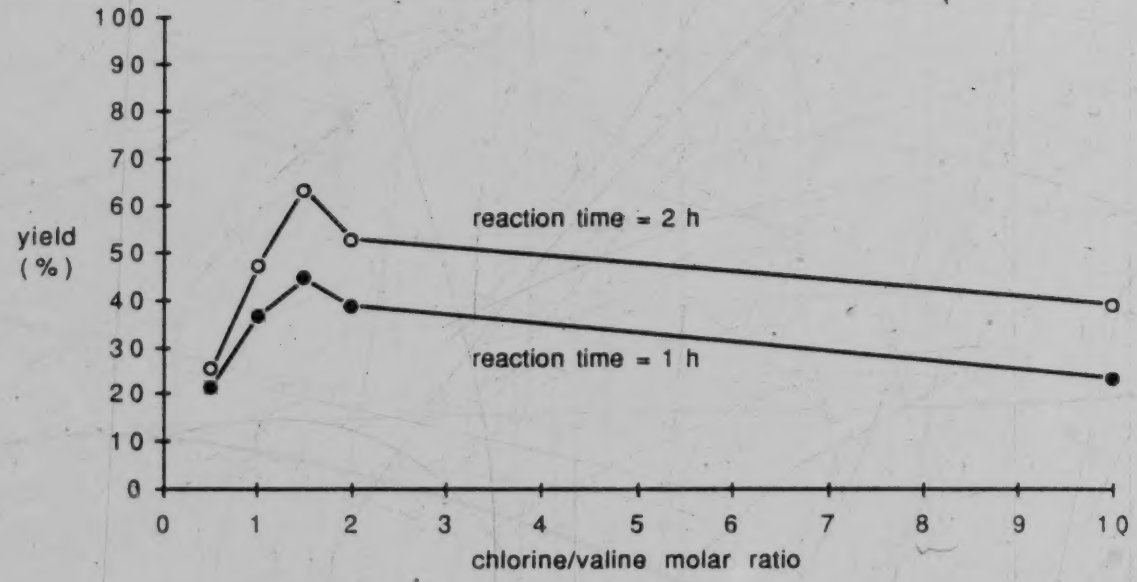


Figure 4.4 Effect of reaction time on isobutyraldehyde formation at varying chlorine/valine molar ratios and pH=8. Theoretical yield of isobutyraldehyde under experimental conditions = 72 µg/L.

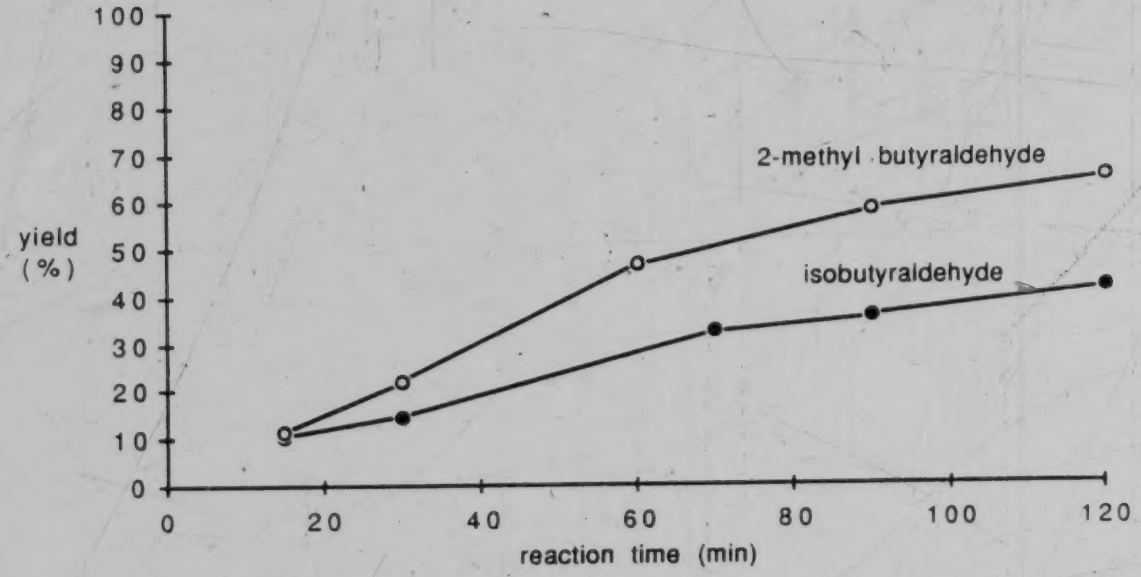


Figure 4.5 Effect of reaction time on aldehyde formation from reaction of amino acids with chlorine at 1:1 molar ratio and pH 8. Theoretical yields under experimental condition: 2-methyl butyraldehyde = 86 µg/L, isobutyraldehyde = 72 µg/L

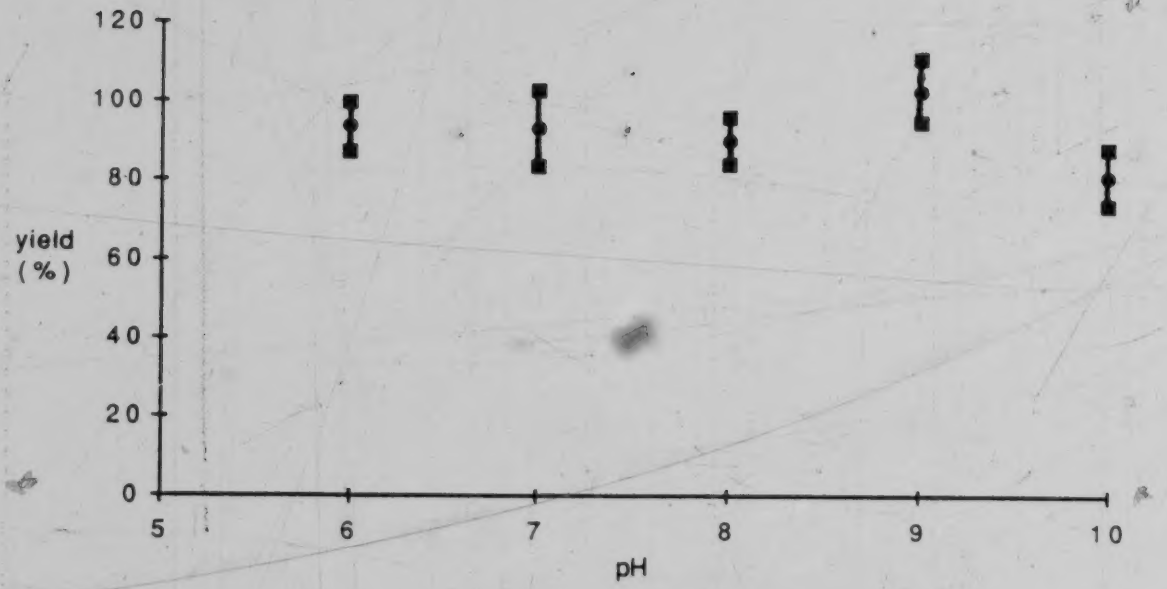


Figure 4.6 Effect of pH on isovaleraldehyde formation from reaction of leucine with chlorine. Reaction time = 2 h, chlorine/leucine molar ratio = 1.5. Error bars represent standard deviation. Theoretical yield of isovaleraldehyde under experimental conditions = 86 µg/L.

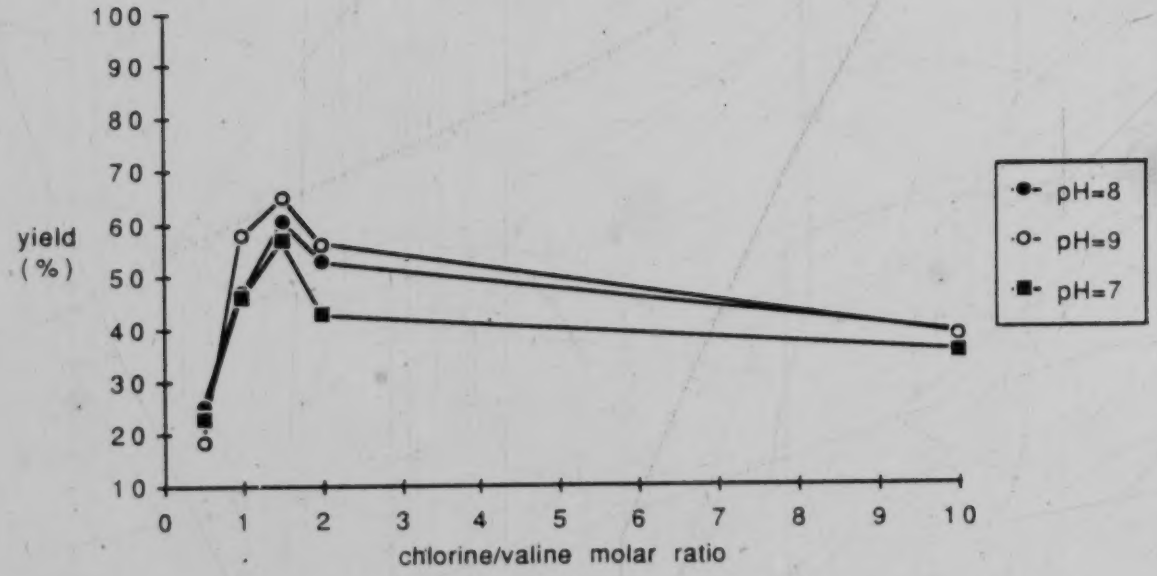


Figure 4.7 Formation of isobutyraldehyde with increasing chlorine dosage at pH 7, 8 and 9. Reaction time = 2 h. Theoretical yield of isobutyraldehyde under experimental conditions = 72 µg/L.

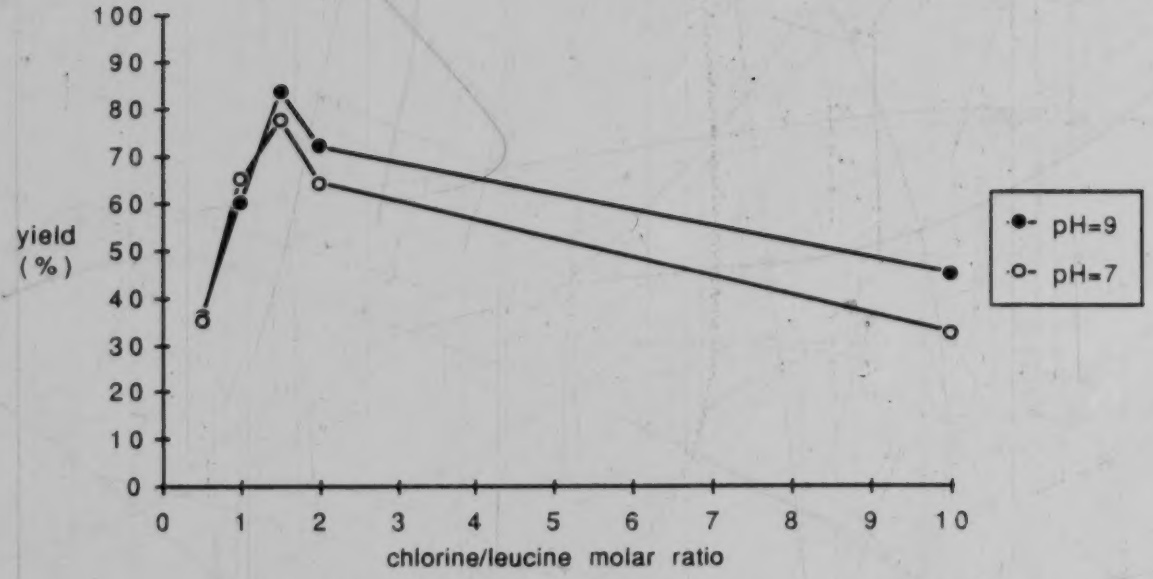


Figure 4.8 Formation of isovaleraldehyde with increasing chlorine dosages at pH 7 and 9. Reaction time = 2 h. Theoretical yield under experimental conditions for isovaleraldehyde = 86 µg/L.

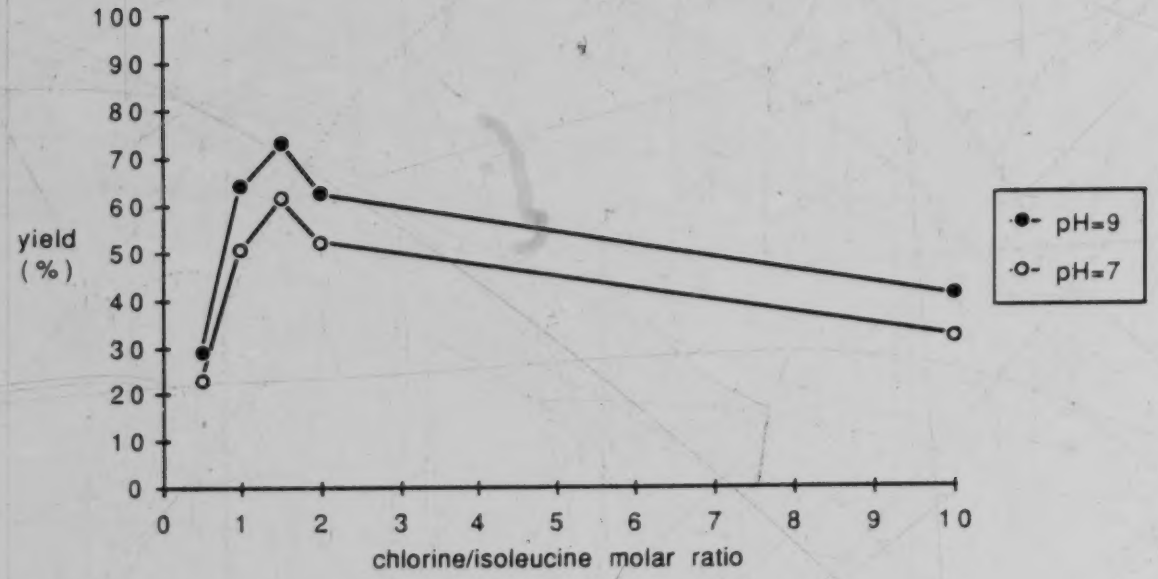


Figure 4.9 Formation of 2-methyl butyraldehyde with increasing chlorine dosages at pH 7 and 9. Reaction time = 2 h. theoretical yield under experimental conditions for 2-methyl butyraldehyde = 86 µg/L.

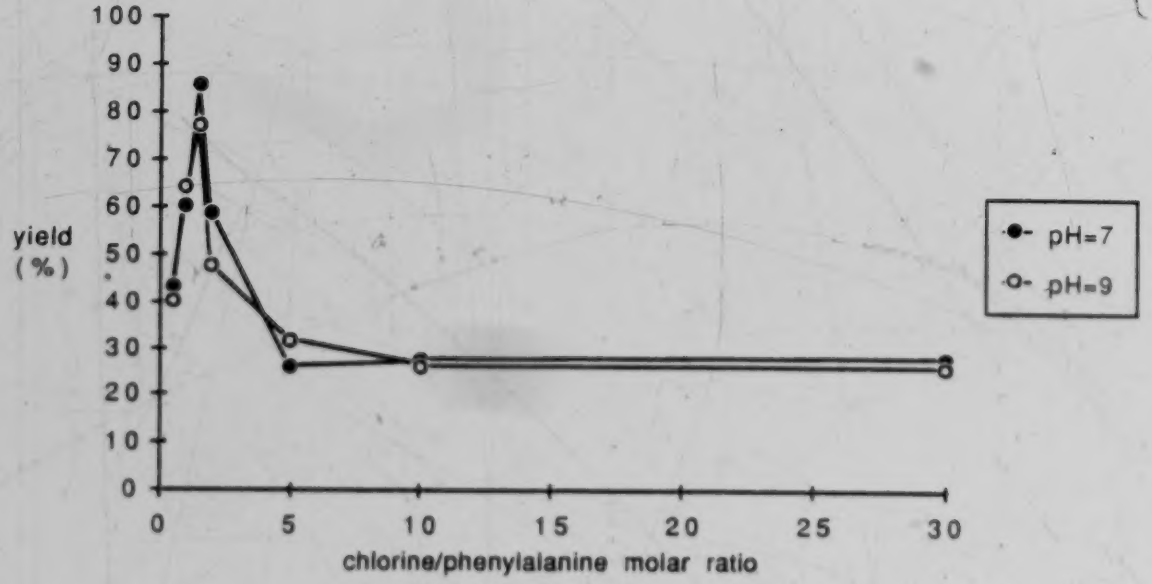


Figure 4.10 Formation of phenylacetaldehyde from reaction of phenylalanine with increasing chlorine dosages at pH 7 and 9. Theoretical yield of phenylacetaldehyde under experimental conditions = 12 mg/L.

Table 4.2 Results from the 2^3 factorial design experiment for chlorination of leucine. Effects presented as µg/L.

Effect	effect+standard error
average	37.60 ±1.67
main effects:	
Time	15.49 ±3.31
Temperature	53.94 ±3.31
pH	5.09 ±3.31
two factor interactions:	
Time x Temp.	8.05 ±3.31
Time x pH	-4.82 ±3.31
Temp. x pH	2.32 ±3.31
three factor interaction:	
Time x Temp. x pH	-4.45 ±3.31

Levels	low	high
Time (min.)	30	120
Temp. (C)	4	20
pH	7	9

4.2.2 Experiments with monochloramine.

4.2.2.1 Aldehyde yield. Monochloramine was also found to convert amino acids to their corresponding aldehydes. The plots of percent theoretical yield of aldehydes versus dosed monochloramine, expressed as monochloramine/amino acid molar ratio (Figure 4.11) and versus reaction time (Figure 4.12) show that under similar experimental conditions, the yields are much smaller than those obtained from reactions with chlorine. Monochloramine gave only 3% of maximum theoretical yield of isovaleraldehyde at a molar ratio of 2, reaction time of 2 hours and the pH of 7. For the same reaction conditions chlorine yielded 60% isovaleraldehyde.

4.2.2.2 Monochloramine/amino acid molar ratio. Aldehyde yields also increase with increasing dosage of monochloramine. As demonstrated in Figures 4.11, 4.16 and 4.17, the highest aldehyde yields were observed at the monochloramine/amino acid molar ratio of 10 which was the maximum ratio applied.

4.2.2.3 Reaction time. To demonstrate the effect of reaction time on aldehyde formation a leucine solution was treated with increasing amounts of monochloramine for 2 and 24 hours. The results presented in Figure 4.13 show that aldehyde levels increased significantly over this range of reaction times. For example, at the given reaction conditions the maximum yield of isovaleraldehyde changed from 10 to 70%.

4.2.2.4 Reaction pH. Figure 4.14 presents results for the yield of isovaleraldehyde at pH 6, 7, 8, 9 and 10 found after 24 hour reaction of monochloramine with leucine at a molar ratio of 10. Maximum yield of isovaleraldehyde was observed at pH 7. The yields decrease substantially with increasing pH (Figure 4.14, 4.15, 4.16). Similar results were obtained from experiments with phenylalanine (Figure 4.17).

4.2.2.5 Combination of factors. Figure 4.15 shows yields of isovaleraldehyde obtained after reaction times of 6, 12 and 24 hours when leucine was treated with a 10 times higher amount of monochloramine at pH 7, 8 and 10. These results show that a long reaction time (24 h) and neutral pH (pH 7) are necessary for the maximum conversion of amino acids to aldehydes.

Results obtained from a factorial design experiment are presented in Table 4.3. These results show that pH and temperature are the most important factors affecting aldehyde formation. Reaction time is another important factor, however the two factor interaction of pH and reaction time suggests that pH has a stronger effect. High effects of two factor interactions are due to their main effects. The calculated effect of molar ratio was insignificant for this experimental design. Calculations of the effects are presented in Appendix 3.C.

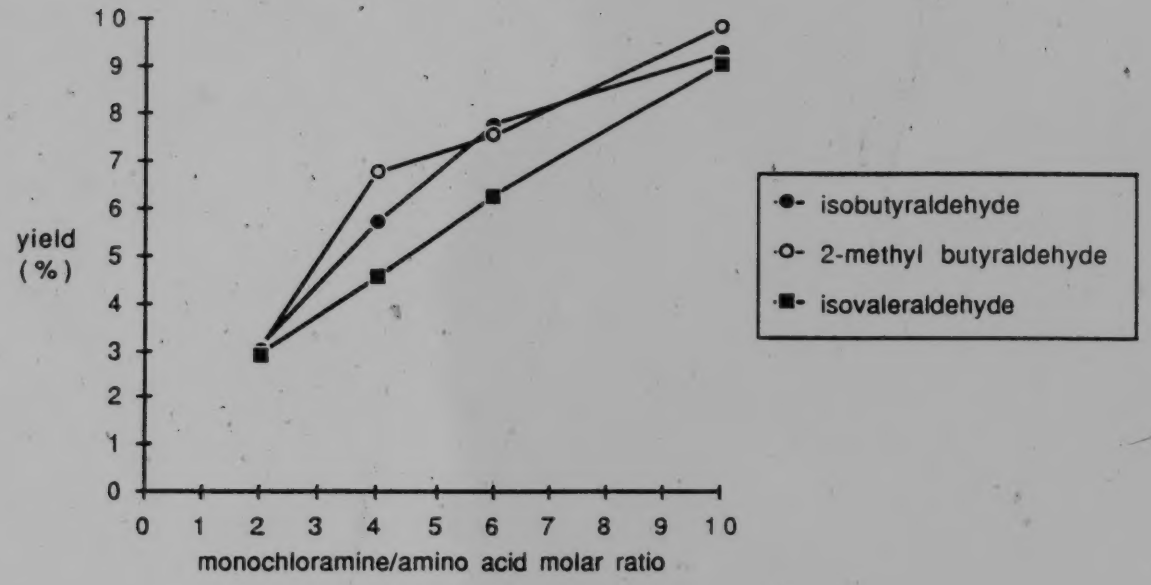


Figure 4.11 Formation of aldehydes at increasing monochloramine/amino acid molar ratios. Reaction time = 2 h, pH = 7. Theoretial yield under experimental conditions : isobutyraldehyde = 72 µg/L, isovaleraldehyde = 86 µg/L, 2-methyl butyraldehyde = 86 µg/L.

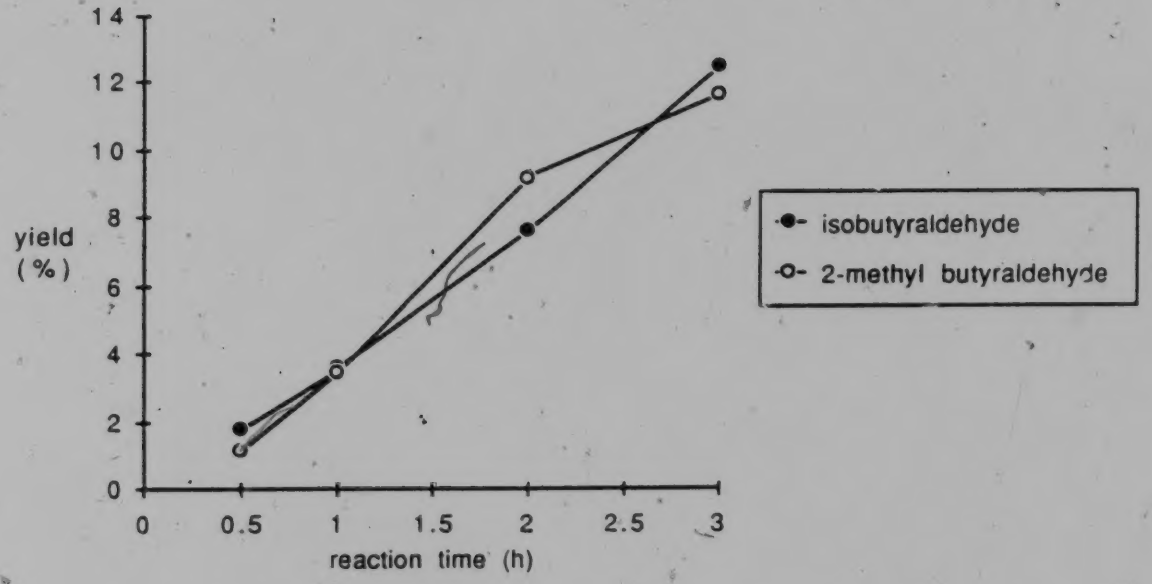


Figure 4.12 Formation of aldehydes with increasing reaction time at pH 7 and monochloramine/amino acid molar ratio = 4. Theoretical yield under experimental conditions: isobutyraldehyde = 72 µg/L, 2-methyl butyraldehyde = 86 µg/L.

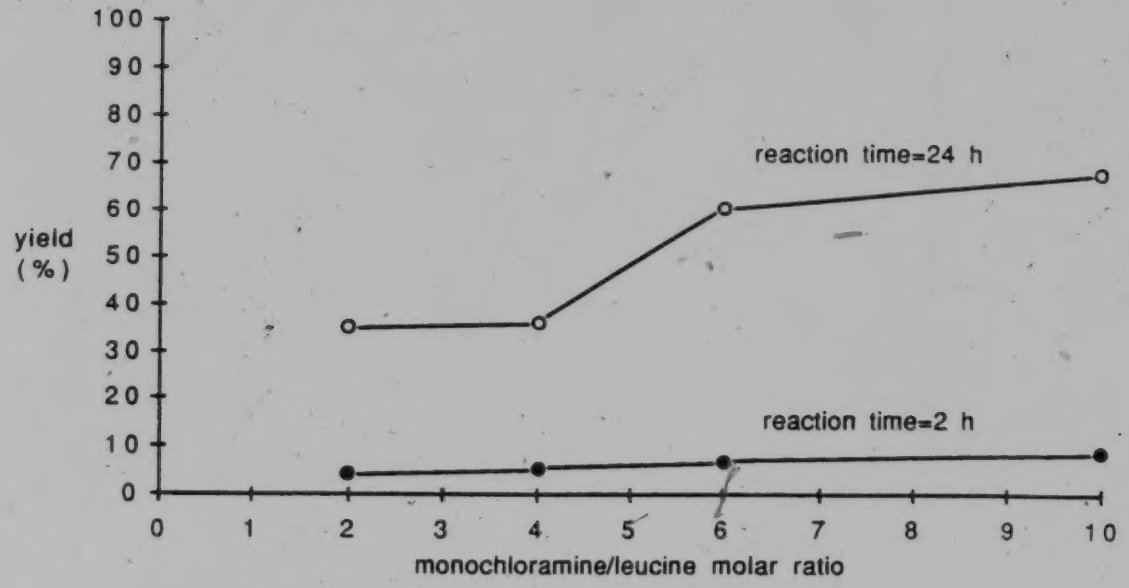


Figure 4.13 Effect of reaction time at pH 7 on isovaleraldehyde formation. From monochloramine/leucine reaction. Theoretical yield of isovaleraldehyde under experimental conditions = 86 µg/L.

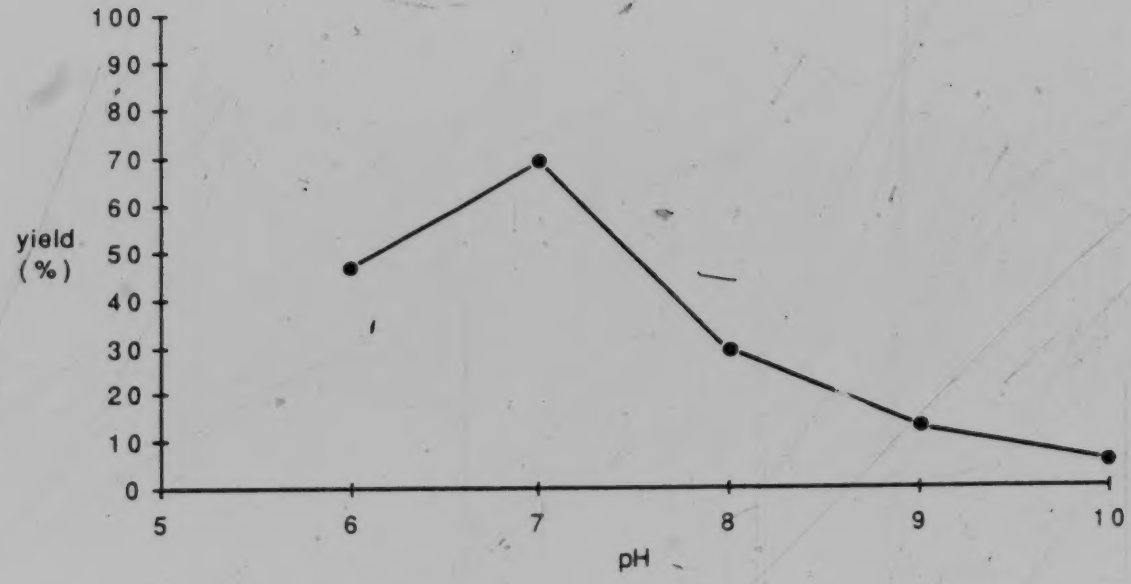


Figure 4.14 Effect of pH on isovaleraldehyde yield from reaction of leucine with monochloramine. Theoretical yield of isovaleraldehyde under experimental conditions=86µg/L.

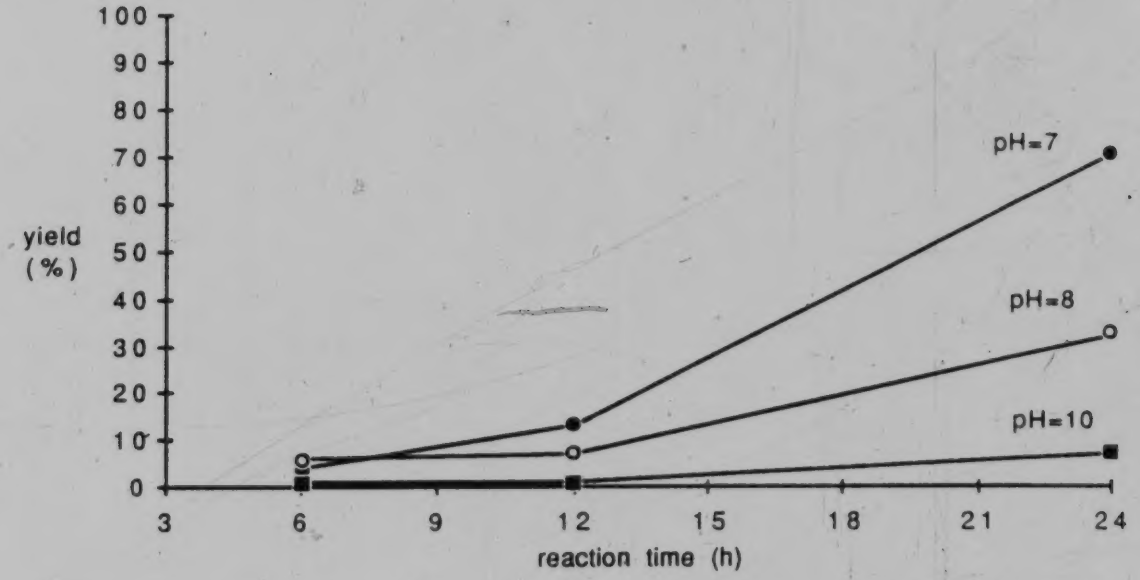


Figure 4.15 Effect of pH and reaction time on formation of isovaleraldehyde from monochloramine/leucine reaction at molar ratio of 10. Theoretical yield of isovaleraldehyde under experimental conditions=86 µg/L.

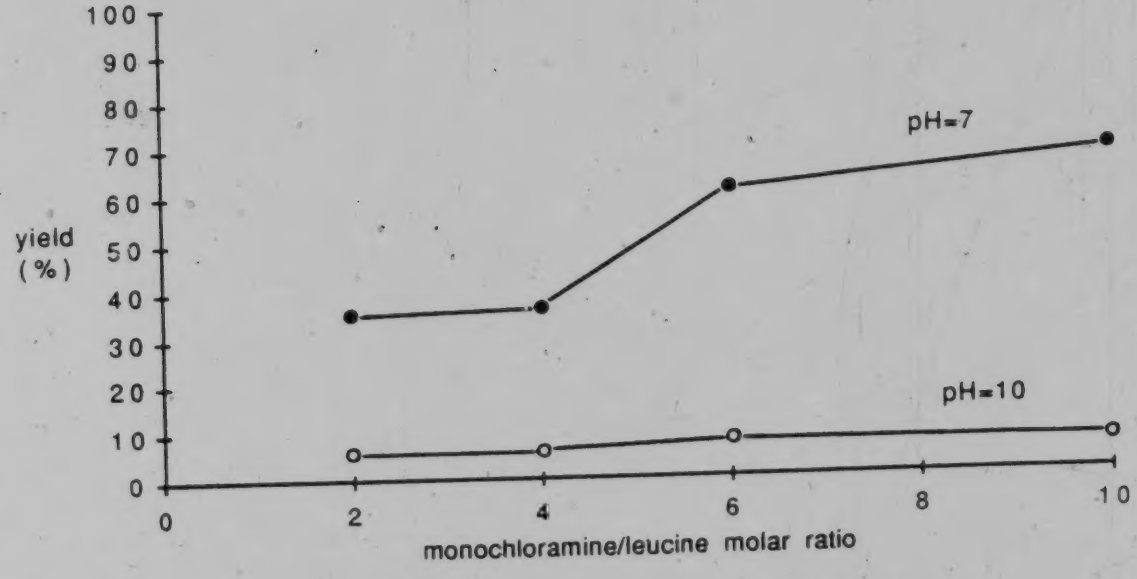


Figure 4.16 Effect of pH and monochloramine dosage on isovaleraldehyde formation. Reaction time=24 h. Theoretical yield of sovaleraldehyde under experimental conditions=86 µg/L.

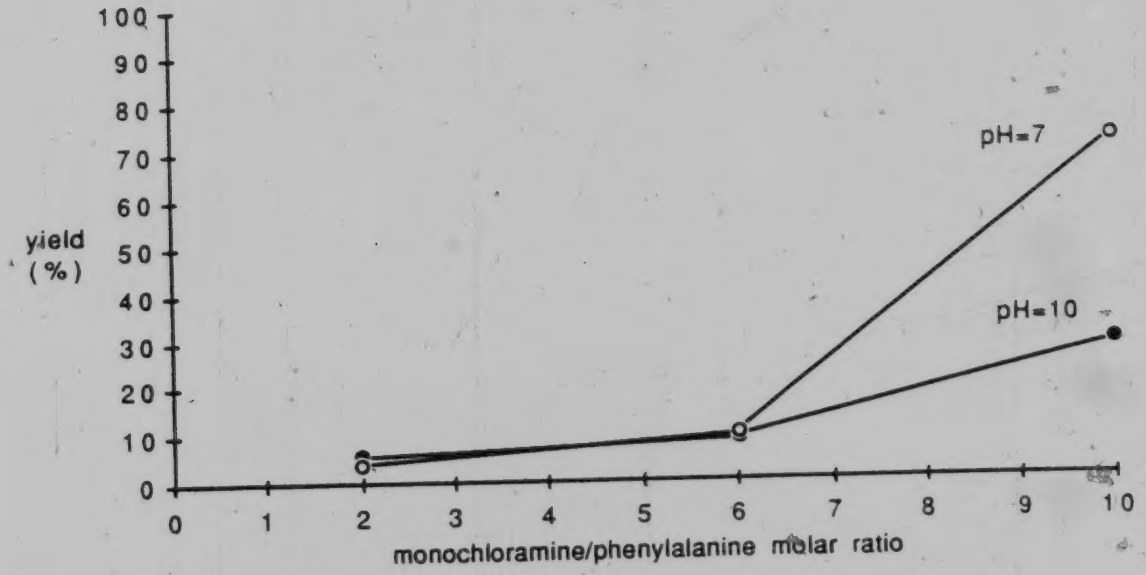


Figure 4.17 Effect of pH and monochloramine dosage on phenylacetaldehyde formation. Reaction time=40 h. Theoretical yield under experimental conditions for phenylacetaldehyde = 12 mg/L.

Table 4.3 Results from the 2 factorial design experiment for chloramination of leucine

CALCULATED EFFECTS

EFFECT	effect ±standard error
average	9.8 ± 0.7
main effects:	
pH	-14.4 ± 0.7
molar ratio	0.5 ± 0.7
Temperature	16.7 ± 0.7
Time	9.7 ± 0.7
two factor interactions:	
pH x molar ratio	-0.3 ± 0.7
pH x Temperature	-14.4 ± 0.7
pH x Time	-9.4 ± 0.7
molar ratio x Temperature	0.3 ± 0.7
molar ratio x Time	0.6 ± 0.7
Temperature x Time	8.8 ± 0.7
three factor interactions:	
pH x molar ratio x Temperature	-0.2 ± 0.7
pH x molar ratio x Time	-0.7 ± 0.7
pH x Temperature x Time	-9.0 ± 0.7
molar ratio x Temperature x Time	0.3 ± 0.7
four factor interaction:	
pH x molar ratio x Temperature x Time	-9 ± 0.7

LEVELS	-	+
A. pH	7	10
B. monochloramine/leucine molar ratio	5	10
C. Temperature (C)	4	20
D. Time (h)	5	24

4.2.3 Experiments with chlorine dioxide.

Chlorine dioxide at a concentration of 900 mg/L used for a preliminary experiment (Exp.1) contained 120 mg Cl_2/L. For subsequent experiments (Exp.2-4) a solution of chlorine dioxide at a concentration of 1000 mg/L containing 46 mg Cl_2/L was used. Chlorine-dioxide free of chlorine was not available. Therefore, the chlorine content must be taken under consideration in evaluating reaction products. At the molar ratios of chlorine dioxide to amino acids ranging from 0.5 to 79.0, the molar ratios for chlorine ranged from 0.03 to 3.0.

Results obtained from experiments are summarized in Table 4.4. and they are plotted in Figures 4.18 to 4.20. For comparative purposes these results are also plotted in Figures 4.21, 4.22 and 4.23 together with the results obtained from reactions of corresponding amino acids with chlorine.

Table 4.4 Results form chlorine dioxide/amino acid experiments.

ClO_2/amino acid molar ratio	Cl_2/amino acid molar ratio	% theoretical yield
(Experiment 1, with leucine, pH=7)		
0.5	0.08	5
1.0	0.17	7.2
1.5	0.25	8.5
2.0	0.33	12
2.5	0.42	18
3.0	0.51	18
3.5	0.60	25.5
4.0	0.68	26.6
(Experiment 2, with leucine, pH=9)		
8.0	0.3	15.8
15.8	0.6	25.1
47.4	1.8	84.9
79.0	3.0	77.8
(Experiment 3, with leucine, pH=7)		
0.8	0.03	0.3
1.6	0.06	2.1
3.2	0.12	4.9
9.5	0.4	15.1
16.0	1.3	51.1
(Experiment 4, with phenylalanine, pH=7)		
5	0.2	7.5
10	0.4	14
20	0.9	32.3
50	2.2	55
100	4.0	20

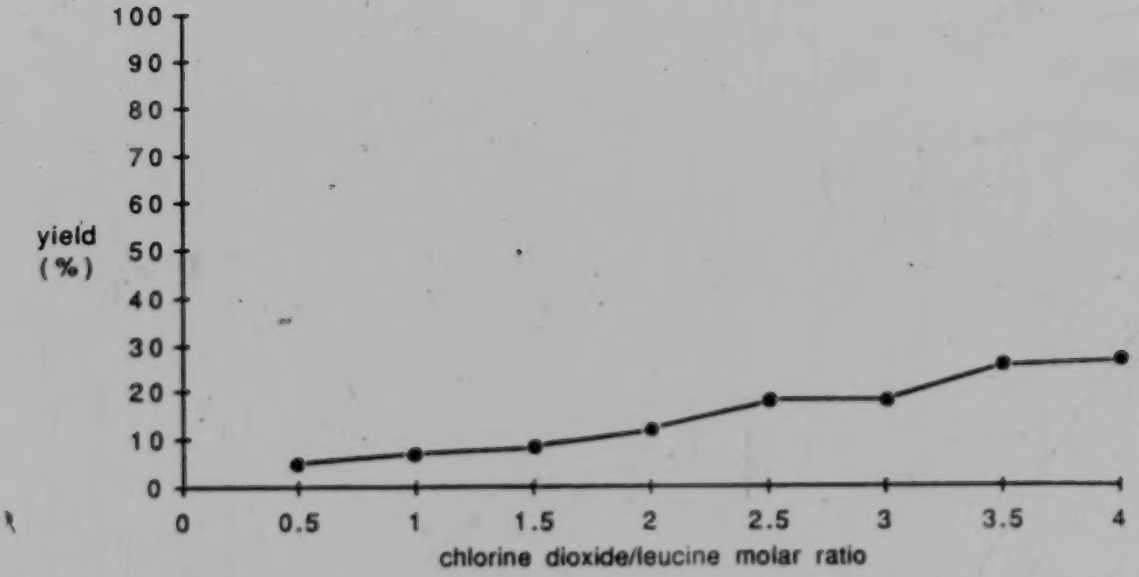


Figure 4.18 Isovaleraldehyde yields from reactions of leucine with chlorine dioxide containing 13% chlorine at pH 7. Theoretical yield of isovaleraldehyde under experimental conditions = 86 µg/L.

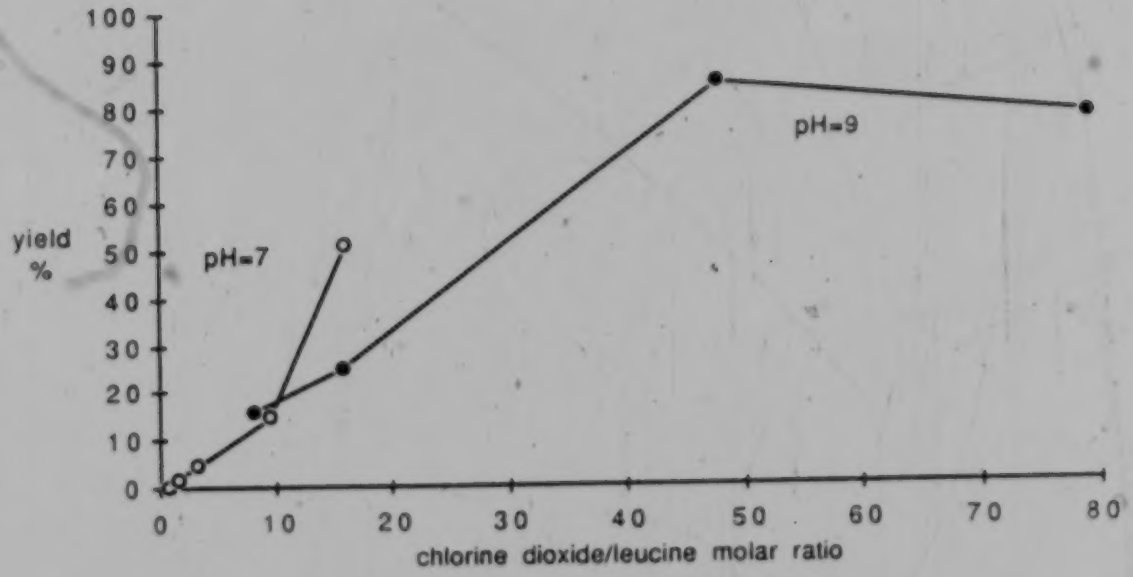


Figure 4.19 Isovaleraldehyde yields from reactions of leucine with chlorine dioxide containing 4.6% chlorine at pH 7 and 9. Theoretical yield under experimental conditions for isovaleraldehyde = 86 µg/L.

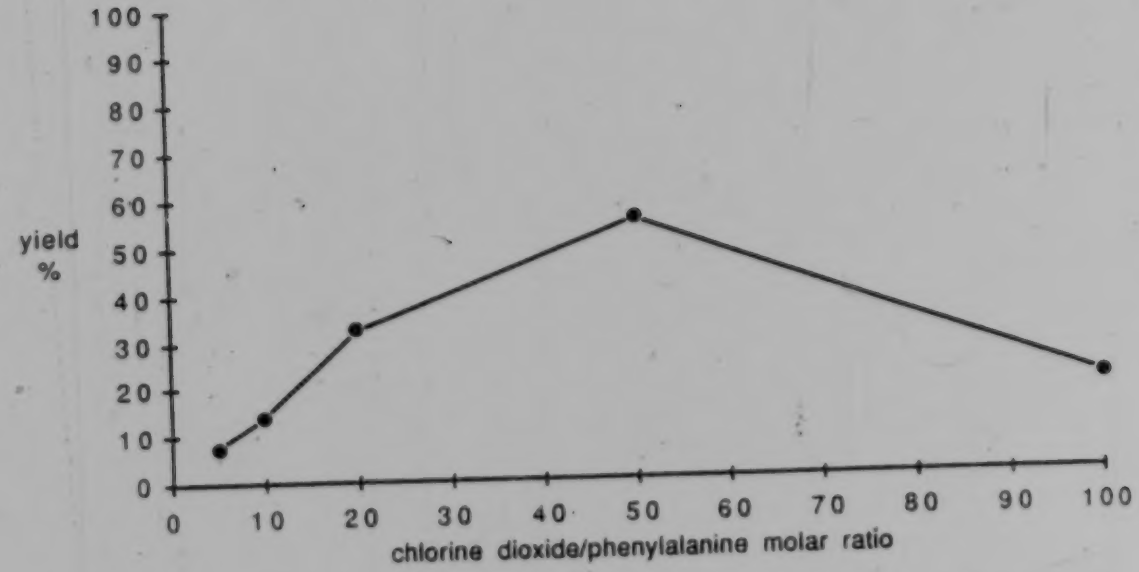


Figure 4.20 Phenylacetaldehyde yields from reactions of phenylalanine with chlorine dioxide containing 4.6% chlorine at pH 7. Theoretical yield under experimental conditions for phenylacetaldehyde = 12 mg/L.

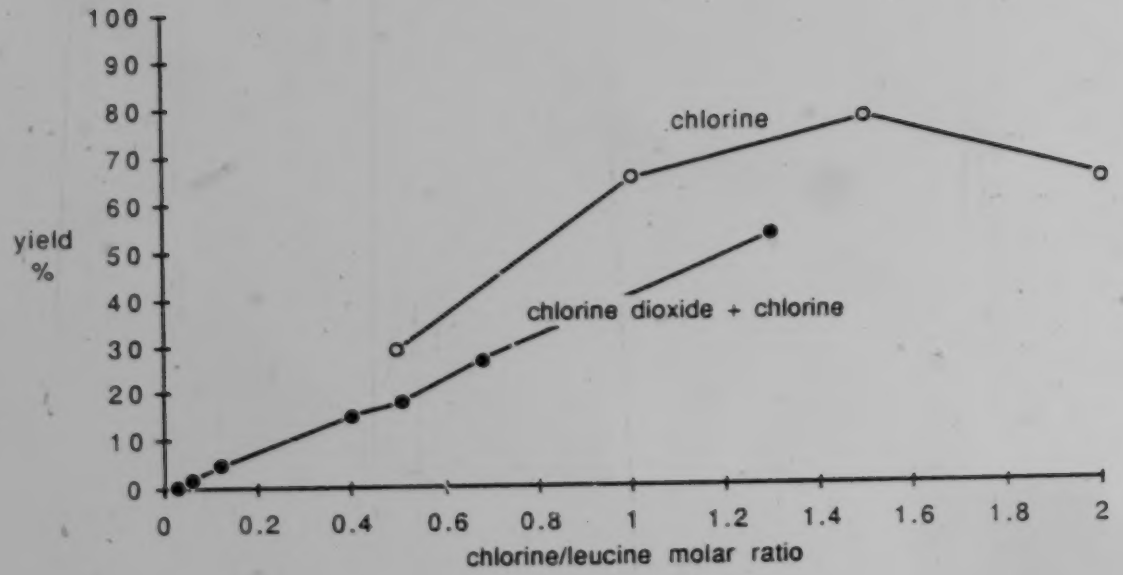


Figure 4.21 Comparison of isovaleraldehyde yields obtained from reactions of leucine with chlorine and with chlorine dioxide containing 4.6% chlorine for 2 hours at pH 9.

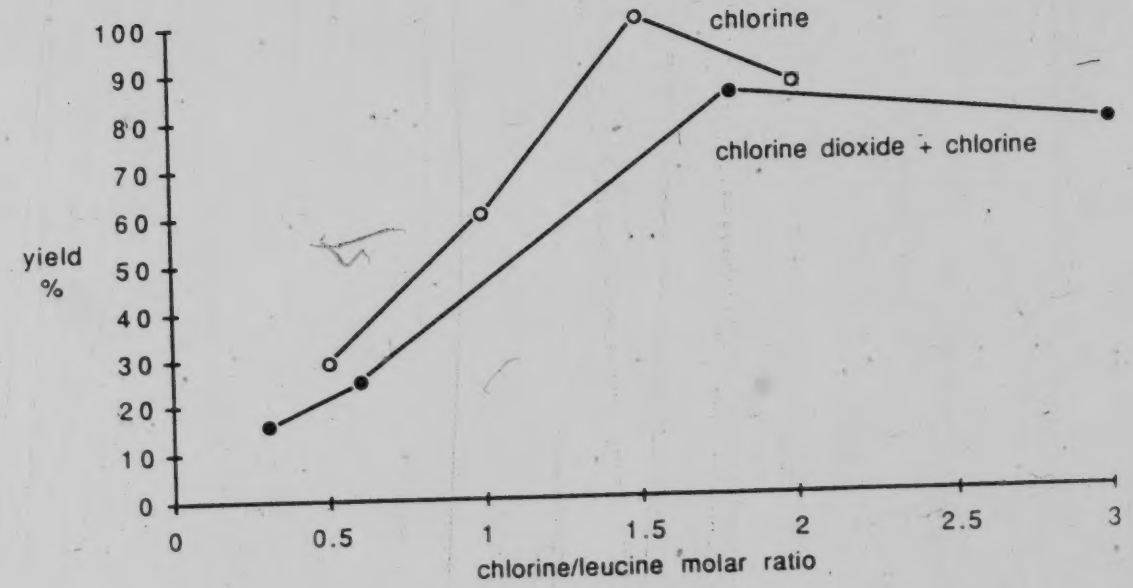


Figure 4.22 Comparison of isovaleraldehyde yields obtained from reactions of leucine with chlorine and with chlorine dioxide containing 4.6% chlorine for 2 hours at pH 9.

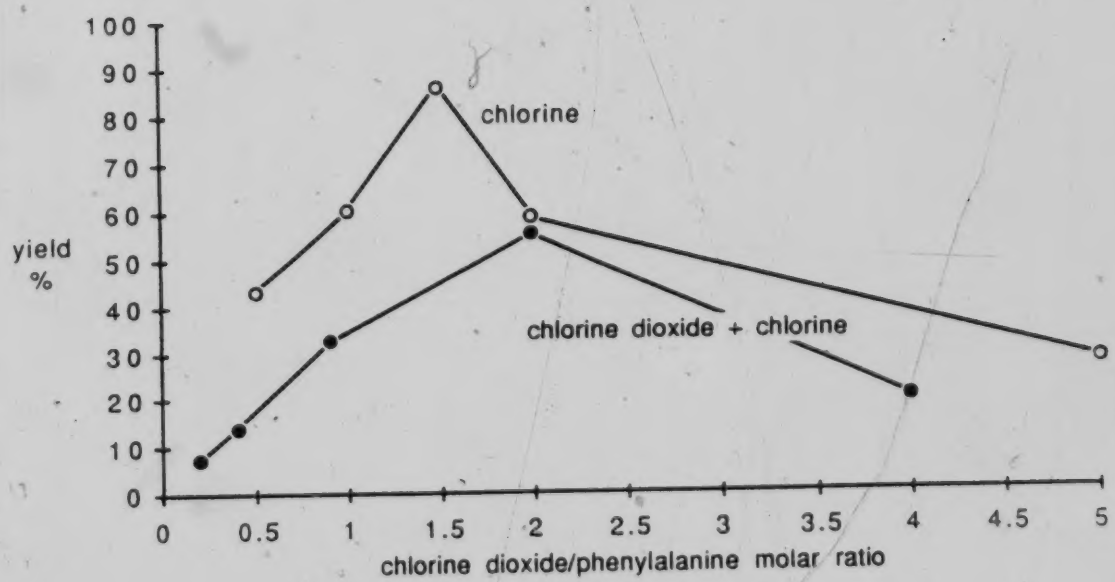


Figure 4.23 Comparison of phenylacetaldehyde yields obtained from reactions of phenylalanine with chlorine and with chlorine dioxide containing 4.6% chlorine for 2 hours at pH 7.

4.2.4 Experiments with ozone.

4.2.4.1 Aldehyde yield. Ozone was found to yield very low levels of aldehydes from amino acids, leucine and phenylalanine. The highest yields of isovaleraldehyde and phenylacetaldehyde at the given experimental conditions were 0.95% and 2.5% respectively.

4.2.4.2 Combination of factors. Figure 4.24 shows that the yields increase with ozone dosage in range of ozone/leucine molar ratio of 0.5 to 5.0. Reactions were very fast. The highest isovaleraldehyde concentration was obtained for the shortest contact time of 5 min.

Figure 4.25 shows phenylacetaldehyde yields detected after 30 min reaction time at pH 7 and 9. No effect of pH on aldehyde formation was observed. However, very low concentrations, close to the detection limits, obtained from these experiments are more susceptible to analytical errors making the results difficult to interpret. This is particularly evident with the phenylacetaldehyde data. The high variability of phenylacetaldehyde results are also due to the occasional presence of an impurity in water used for experiments. During chromatographic analysis, this impurity was co-eluting with the phenylacetaldehyde. Different methods such as boiling of water or passing it through an ion exchange resin were unsuccessful in eliminating the co-eluting impurity. The presence of this impurity was noticed during method preparation when chromatographic analysis of blanks were performed.

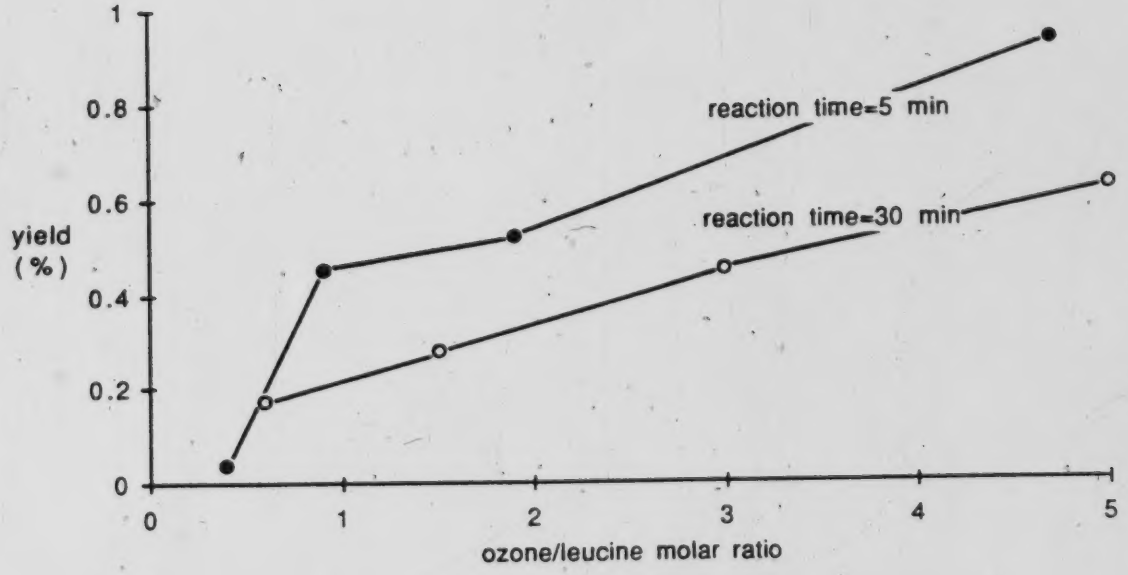


Figure 4.24 Effect of reaction time and ozone dosage on isovaleraldehyde formation at pH 7. Theoretical yield under experimental conditions=0.86 mg/L.

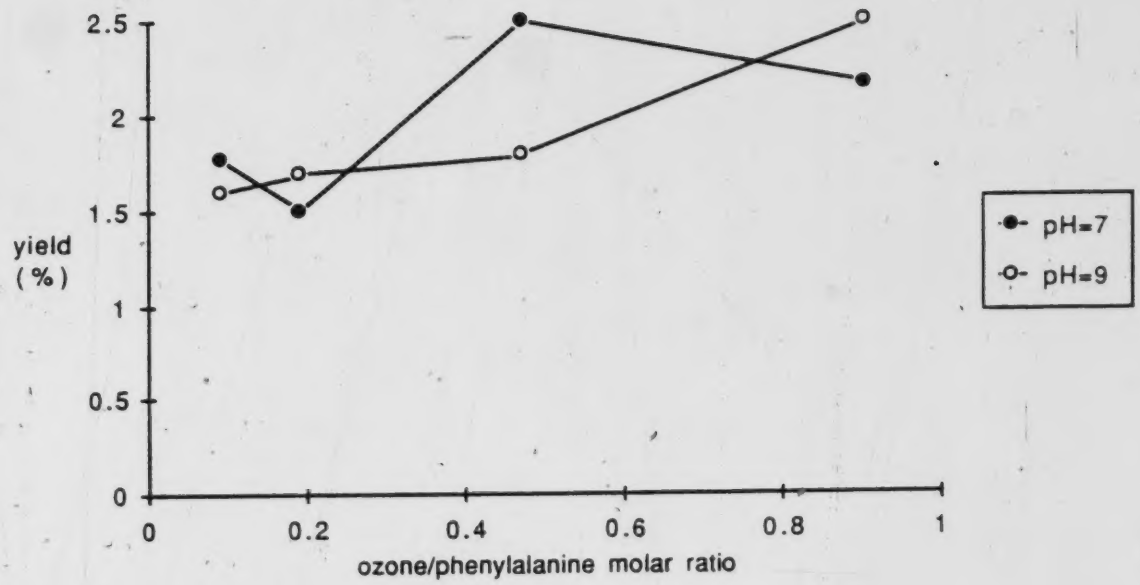


Figure 4.25 Effect of ozone dosage and pH on phenylacetaldehyde formation.
Theoretical yield=120 mg/L

4.3 Analysis of water from swimming pools.

In order to test the expectation that chlorination of water containing amino acids should yield the odorous aldehydes, duplicate samples of water from three municipal swimming pools were collected and analyzed. These analyses revealed aldehyde concentrations ranging from 1 to 7 µg/L for isovaleraldehyde, 6 to 12 µg/L for isobutyraldehyde and 1 to 22 µg/L for 2-methyl butyraldehyde. The results are summarized in Table 4.5. The samples could not be analyzed for phenylacetaldehyde because of the inadequate sensitivity of the method used for its analysis.

Table 4.5 Summary of the results from analysis of swimming pool water.

Aldehyde	concentration µg/L #1	#2	average	swimming pool
isobutyraldehyde	11.6	-	11.6	Hardisty
	5.5	11.3	8.4	Mill Woods
	10.8	7.1	9.0	Londondery
2-methyl butyraldehyde	6.7	6.2	6.5	Hardisty
	1.3	3.1	2.2	Mill Woods
	22.4	19.8	21.1	Londondery
isovaleraldehyde	6.5	-	6.5	Hardisty
	3.7	3.6	3.7	Mill Woods
	1.4	0.9	1.2	Londondery

5. DISCUSSION

5.1 Formation of odorous aldehydes from reactions of disinfectants with amino acids.

This research demonstrated that the odorous aldehydes: isobutyraldehyde, 2-methyl butyraldehyde, isovaleraldehyde and phenylacetaldehyde can be formed under conditions occuring in water treatment from reactions of certain disinfecting agents with corresponding amino acids: valine, isoleucine, leucine and phenylalanine.

Amino acids, the source of odorous aldehydes are virtually unavoidable in a surface water supply. Therefore, reactions which convert amino acids into aldehydes creating a taste and odour problem are of a major practical significance.

Quantitative analysis of odorous aldehydes, performed with sufficient sensitivity to detect these compounds around their threshold odour levels, allowed the study of their yields under relevant water treatment conditions.

5.1.1 Formation of aldehydes from chlorine/amino acid reactions.

This study confirmed that chlorine readily converts amino acids to their corresponding aldehydes. Similar conversions for other amino acids have been well documented in the literature.

Physicochemical factors affecting aldehyde yields include: temperature, reaction time and relative ratios of chlorine to amino acids. As demonstrated by the factorial design experiment, temperature has the greatest effect on reaction rate. At 4°C only minute concentrations of isovaleraldehyde were detected. Reactions at 20°C are rapid. For example, 11% of the theoretical yield of isobutyraldehyde was obtained after 15 minutes for the chlorine/valine molar ratio of 1:1. Experiments carried out by Madec et al. (1985) on samples of sea water treated with 1 mg/L of chlorine

showed that about 5% of chlorine, which dissipated during the first 3 minutes, was consumed by the dissolved free amino acids, the depletion of which was about 50%. Antelo et al. (1988) claim that the reaction of chlorine with serine resulting in the formation of chlorinated amino acid was instantaneous.

Aldehyde yields were found to increase with time. Two hour reaction time showed quantitative conversion of leucine to isovaleraldehyde at a 1.5 chlorine/leucine molar ratio. To the contrary, Le Cloirec and Martin (1985) observed a gradual decrease of acetaldehyde during a 2.5 hour reaction of chlorine with alanine. They explain this decrease by subsequent oxidation of aldehydes to nitriles by monochloramine which is also formed in the reaction medium. Their observations are based on reactions in an unbuffered solution.

The results of formation of aldehydes at different chlorine dosages are completely in accordance with those reported by Le Cloirec and Martin (1985). The extent of the aldehyde production depends upon chlorine/amino acid molar ratio, with the maximum aldehyde yield at 1.5. However, increasing chlorine dosages (molar ratio above 5) did not have any further effect on aldehyde levels (Figure 4.10).

The pathway of chlorine/amino acid reactions is well documented in the literature (Section 2.5.2.1). Chlorine reacts with amino acids to form unstable chloroamino acids. If the chlorine/amino acid ratio is high, dichloroamino acids are mainly formed prior to the formation of nitriles. If the ratio is low, monochloroamino acids are formed which subsequently decompose to aldehydes. This study suggests that the chlorine/amino acid molar ratio at which monochloroamino acids are formed is below approximately 1.5. Above this level aldehyde concentrations decrease, presumably with increasing concentration of nitriles.

Le Cloirec and Martin (1985) discuss the possibility of another reaction pathway. They suggest that chlorine forms monochloroamino acids which decompose to aldehydes with subsequent oxidation to nitriles by monochloroamine (NH_2Cl) which is also formed during the course of initial reaction.

The literature suggests that pH affects the formation of chlorinated amino acids. At acidic pH mainly dichloroamino acids are formed which lead to nitriles. At alkaline pH, monochloroamino acids prevail with decomposition forming aldehydes. In this study, the effect of pH ranging from 6 to 10 was found to be negligible. These results do not necessary contradict the qualitative statements stressing the importance of pH on aldehyde yield often found in the literature where most of the experiments were conducted at extreme pH values. Antelo et al. (1988) studied the effects of hydrogen ion concentrations over the pH range of 6-9 on decomposition of N-chloro-L-serine and concluded that over this range the decomposition is independent of pH. At pH <5 and pH>9 the rate of decomposition is greater.

The reactivity of amino acids differs slightly. The most reactive with respect to aldehyde yields appears to be leucine and phenylalanine followed by isoleucine and valine. For example, the percent theoretical yields for phenylacetaldehyde, isovaleraldehyde, 2-methyl butyraldehyde and isobutyraldehyde at pH 7 and the optimum molar ratio of 1.5, were 87, 77, 61 and 57 respectively. However, when comparing yields of phenylacetaldehyde to yields of the aliphatic aldehydes, it should be taken under consideration that different methods were used for their analysis and quantitation. The highest reactivity of leucine (among three aliphatic aldehydes) with respect to isovaleraldehyde formation was confirmed for all other experiments.

The reactivity of amino acids towards chlorine is, as shown, affected by physicochemical properties occurring during water chlorination but is also dependent upon the nature of amino group. While β and γ free amino acids yield stable halogenocompounds, only the α amino acids yield unstable haloamines which decompose rapidly (Madec et al., 1985).

5.1.2 Formation of aldehydes from monochloramine/amino acid reactions.

The odorous aldehydes can also be formed upon chloramination of amino acids. Results obtained with monochloramine revealed that a higher oxidant/amino acid molar ratio than observed with chlorine will maximize aldehyde formation. A much longer contact time is also required for quantitative conversion of amino acids to aldehydes.

Temperature, as well as pH, were found to be major factors influencing the formation of aldehydes with monochloramine. Temperature of 4°C lowered the aldehyde yields to trace quantities regardless of other reaction conditions.

Maximum yields obtained at pH 7 decreased gradually with increasing pH. There is a striking contrast in the way chloramination and chlorination respond to pH. In the case of chlorination, pH was found to have negligible effect on aldehyde formation whereas pH is a major factor affecting aldehyde yield if monochloramine is used as oxidant. This dependence of pH on aldehyde formation is difficult to explain. As mentioned previously, the literature concerning these reactions is meagre.

Mainly N-chloro compounds have been recognized in the literature as by-products of amino acid monochloramination. The results of this study suggest however that N-chloro compounds are by-products but not end-products of the reactions if sufficient reaction time is ensured.

There appears to be two general steps of aldehyde formation upon chloramination. The first is chlorine transfer from monochloramine to amino acids and the second is decomposition of monochloroamino acids.

Transfer of active chlorine from monochloramine to amino acids and a subsequent formation of chlorinated species has been well documented. The main mechanisms of chlorine transfer is a direct transfer. However, as suggested by Isaac and Morris (1980), hydrolysis should also be considered for low (10^{-5} mM) concentrations of substrates. Although Isaac and Morris (1983) state that this step is not dependent on a hydrogen ion concentration in

the pH range of environmental significance (pH 6.5-8.5), Snyder and Morgerum (1981) found that the rate constant for this reaction decreases drastically at pH 10.

It is known that pH affects the stability of monochloramine in aqueous solution. The stability of monochloramine increases with increasing pH. The half life for monochloramine at pH 7 is 17.8 hours. At pH 9 the oxidant is extremely stable having a half life of 770 hours. Jacangelo et al., (1987) found that the decay of monochloramine at pH 7 and a temperature of 25°C during 10-20 minutes was about 2%. Reaction temperature in the range 4-20°C would not have any effect on monochloramine stability. The influence of pH on monochloramine stability is perhaps one of the possible explanations for the significant effect that pH has on aldehyde yields from monochloramine reaction with amino acids. At the current state of knowledge with regard to the chemistry of aldehyde formation from monochloramine/amino acid reactions no conclusions could be drawn as to the exact mechanism of these reactions.

Another parameter that has a substantial effect on aldehyde yields is reaction time. As shown in Figure 4.13, for the molar ratio of 10, the yield of isovaleraldehyde changed from 10 to 70% for 2 and 24 hour reaction times respectively. Aldehyde yields also increased with increasing monochloramine dosage. These results somewhat contradict the findings reported in the literature that monochloramine readily reacts with aldehydes, producing nitriles (Hauser et al., 1930, Le Cloirec and Martin, 1985). No decrease was observed for 24 hour reaction time and increasing monochloramine dosage. At the pH of 7, aldehyde yields increased gradually with time: At the pH of 10, concentrations of aldehydes were very low regardless of the reaction time and monochloramine dosages.

A factorial design experiment demonstrated that temperature, pH and time are important factors influencing the formation of aldehydes. The effect of the molar ratio calculated to be near zero suggesting that this factor is insignificant, contradicted results obtained from one-factor-at a time experiments. This inconsistency is caused by the confounding effect of other variables. Calculation of

this effect was based on 8 results of which only two showed the positive effect of increasing monochloramine dosage. Four of the results obtained at 4°C produced only trace quantities of the aldehyde because of the important effect of temperature. Two out of the remaining four results were obtained from the reactions conducted at 20°C but with a reaction time of 6 hours which was too short to demonstrate a substantial effect of the molar ratio.

5.1.3 Formation of aldehydes from ozone/amino acid reactions.

The odorous aldehydes are also produced from the reactions of amino acids with ozone. The yields obtained in these experiments are very low. For the same reaction conditions (molar ratio 0.9, reaction time 30 min, pH 7) the aromatic phenylalanine gave higher yields (2%) than the aliphatic leucine (0.2%).

Ozone is a very strong oxidizing agent. Its oxidation potential is 2.07 volts as compared to 1.36 volts for chlorine and 1.275 volts for chlorine dioxide. Ozone reacts by two different mechanisms in aqueous solution. At low pH (below 6) it reacts directly as the oxidizing molecule (O_3). At higher pH (above 8) ozone dissociates into hydroxyl free radicals $(OH)^{\bullet}$ which are oxidizing species. Between pH 6 and 8, ozone reacts by either or both pathways (Rice and Gomez-Taylor, 1986). The literature does not provide any specific information regarding aldehyde formation from ozone/amino acid reactions. However, Yamanaka et al. (1979) reports the formation of isobutyraldehyde, isovaleraldehyde, 2-methyl butyraldehyde and phenylacetaldehyde from the reactions of amino acids with hydrogen peroxide. Both ozone and hydrogen peroxide (H_2O_2) generate hydroxyl radicals as oxidizing species. This suggests the possibility that ozone as well as hydrogen peroxide, both of which possess high oxidizing power, react with amino acids through the same mechanism, specifically by deamination and decarboxylation. Katz (1980) and Rice and Gomez-Taylor (1986) mention that amino groups are converted to ammonia and nitrate ion so that deamination proceeds directly through cleavage of the ammonia bond. However,

these reactions are less specific than the chlorine reactions and aldehydes may be only one of a variety of oxidation products. The reaction pathway for aldehyde formation through formation of chlorinated amino acids from chlorine or monochloramine appears to be more efficient.

Higher yields of isovaleraldehyde observed for shorter contact times suggest that aldehydes formed during the course of reaction are being further oxidized by residual ozone. High reactivity of ozone towards aldehydes has been indicated in the literature. Rice and Gomez-Taylor (1986) report that formaldehyde, the ozone oxidation product of many organics, is rapidly oxidized to CO_2 and H_2O upon continued ozonation. The aldehydes, benzaldehyde, isobutyraldehyde, isovaleraldehyde and acetaldehyde are converted to their respective paracids and acids (Falk and Moyer, 1976).

The iodometric procedure used for determining ozone concentrations is considered by some to be inaccurate because of the great number of potential interferences and other complications. There is a 5 to 10% difference between ozone concentrations measured by this and by the colorimetric technique (Smith, 1988) for example. However, this level of inaccuracy would not significantly alter the interpretation of the results obtained in the ozone experiments.

5.1.4 Formation of aldehydes from chlorine dioxide/amino acid reactions.

Chlorine dioxide did not produce aldehydes from their corresponding amino acids in any substantial quantities. As indicated in the literature review section, the majority of researchers working on reactions of chlorine dioxide with amino acids concluded that the simple amino acids, not containing reactive sites such as sulfur, are stable to chlorine dioxide oxidation.

Unfortunately, chlorine dioxide free of chlorine was not available. The experiments were performed with chlorine dioxide containing known amounts of chlorine. The results obtained from

these experiments are plotted along with the results obtained from experiments with chlorine, as aldehyde yields versus chlorine/amino acid molar ratio. Results presented in Figures 4.21-4.23 show that for the same experimental conditions the yields from reactions of chlorine dioxide containing chlorine are not higher than those which were obtained when only chlorine was applied. These results suggest that chlorine dioxide does not react with amino acids and the aldehydes formed are the products of amino acid reaction with the chlorine content. Although traces of phenylacetaldehyde have been reported as a chlorine dioxide/phenylalanine oxidation product by Taymaz at al. (1979) and by Rice and Gomez-Taylor (1986), these reports are not clear about the possibilities of chlorine being present in the chlorine dioxide solution, with the chlorine being responsible for the aldehyde yield observed.

The profiles observed for chlorine and for the chlorine in chlorine dioxide in Figures 4.21, 4.22 and 4.23 are very similar. Specifically, the chlorine in chlorine dioxide profiles suggest the possibility of maximum aldehyde yield near a chlorine/amino acid molar ratio of 1.5. These observations lend further weight to the proposition that the aldehydes observed result from the chlorine contamination in chlorine dioxide rather than from the chlorine dioxide directly.

Chlorine dioxide does not produce N-chlorinated derivatives of amino acids (Rice and Gomez-Taylor, 1986). In the case of chlorine and monochloramine reactions with amino acids, these chlorinated amino acids are intermediate steps towards the aldehyde formation. Evidently, chlorine dioxide does not perform reactions analogous to that of ozone by the "direct" deamination and decarboxylation either. The oxidation potential of chlorine dioxide is lower than that of ozone or even chlorine.

When leucine and phenylalanine were treated with a mixture of chlorine dioxide and chlorine, the yields of isovaleraldehyde and phenylacetaldehyde were observed to be slightly lower than those when only chlorine was applied. In the case of phenylalanine, evidence obtained from the literature suggests the possibility of aromatic ring oxidation by chlorine dioxide with the formation of

alanine. This reaction could lower the phenylalanine content available for chlorination. The possibility of produced aldehyde being oxidized by very high doses of chlorine dioxide should also be considered, although no reports of chlorine dioxide/aldehyde reactions were found in the literature. Of course, it is also possible that the differences could result from an experimental variation. To avoid an additional formation of chlorine in the chlorine dioxide solution upon dilution, very small volumes of concentrated chlorine dioxide/chlorine solutions were added to the samples. Measurement errors usually increase with decreasing volumes of reagents applied.

5.2 Formation of aldehydes under water treatment conditions.

Experimental conditions for this study were similar to those that are normally employed in a full scale water treatment plant. These experiments demonstrated that the odorous aldehydes can be formed under plant conditions from reactions of disinfectants with amino acids present in water supply. Out of four disinfection agents tested, chlorine and monochloramine are capable conversion of amino acids to aldehydes with very high yield.

5.2.1 Formation of aldehydes by chlorination and chloramination.

Chlorine is the most efficient in converting amino acids to the odorous aldehydes. For a given amino acid concentration, temperature and the relative ratio of chlorine to amino acids will have the major effects on aldehyde yield.

Monochloramine is also effective in producing odorous aldehydes from their corresponding amino acids. Longer reaction times and higher monochloramine dosages will maximize aldehyde yields. Monochloramine, a predominant form of combined chlorine, has been used in water disinfection as a source of active chlorine because it is not very volatile and is slow to hydrolize. However,

monochloramine has lower oxidation potential than does chlorine, hence is less effective as oxidant. Longer contact time and higher doses are therefore required to assure the proper disinfection. A general practice of chloramination is to provide and maintain a stable residual throughout a distribution system to the point of consumer use. Therefore, the conditions created for chloramination are also suitable for the conversion of free amino acids into odorous aldehydes.

At the amino acid concentration of 10^{-6} mM, practically any amount of chlorine in the range tested (0.5-30 chlorine/amino acid molar ratio) yielded significant (above their threshold odour level) amounts of aldehydes. At least 20% yield was obtained for isobutyraldehyde and 2-methyl butyraldehyde and 30% for isovaleraldehyde and phenylacetaldehyde when the reaction was performed at room temperature. These results can be related to the amino acids levels that would yield aldehydes above their threshold odour concentrations. For isobutyraldehyde, 20% yield at the valine level of 117 µg/L (0.001 mM) is equivalent to 21.6 µg/L which is about 22 times higher than its threshold odour concentration. Assuming 30% conversion of isovaleraldehyde, a leucine concentration of 1 µg/L yields an isovaleraldehyde concentration around its threshold odour level of 0.2 µg/L. For 2 methyl butyraldehyde whose threshold odour of 12.5 µg/L is the highest of the four aldehydes, 63 µg/L of isoleucine is needed, assuming 30% conversion, to reach its threshold odour level. Similarly, phenylalanine at a concentration of 18 µg/L would yield phenylacetaldehyde at its odour threshold level.

These levels of amino acids, as discussed later, are well within the range that can be encountered in eutrophic waters.

To investigate the possibility of aldehyde formation upon chlorination of organically enriched waters, water samples from swimming pools were analyzed for aldehyde content. The odorous aldehydes were identified in these samples in concentrations above their odour threshold levels. Concentrations of isobutyraldehyde varied between 5.5 and 11.6 µg/L, i.e. 3 to 6 times its threshold odour concentration. Isovaleraldehyde was found in concentrations

ranging from 0.9 to 6.5 which is about 4.5 to 32.5 times its threshold odour level, whereas a 2-methyl butyraldehyde concentration of 22.4 µg/L was twice its threshold odour level. These findings suggest that the odorous aldehydes are significant contributors to the widely recognized "swimming pool" odour.

As with chlorination, yields of aldehydes from the oxidation of amino acids by monochloramine will depend on the molar ratio of amino acid to the oxidant and the susceptibility of amino acids to oxidation relative to other organic compounds present. This in turn will depend upon the chemical composition of disinfected water. In practice, as TOC levels rise, applied disinfectant levels will rise. If the TOC is not primarily composed of amino acids then the monochloramine/amino acid ratios could increase. The highest yield obtained from isovaleraldehyde was 70% for pH 7, with a reaction time of 24 hours and monochloramine/leucine molar ratio 10:1. Such high conversion will not be encountered in waters because of the high dependence of the reaction conditions such as temperature pH and reaction time. The highest yield of isobutyraldehyde obtained at pH 8 was about 30%.

Apparently, variability in amino acid concentrations in disinfected water will be one of the major factors that will determine the occurrence of the odour problem created by formation of aldehydes.

5.2.2 Formation of aldehydes by ozonation.

Ozone oxidizes amino acids to aldehydes with a very small yield under drinking water treatment conditions. Because of the very low yields of aldehydes produced by ozonation of amino acids and the likely subsequent oxidation of aldehydes by residual ozone, levels of aldehydes sufficient to result in odour problems are unlikely to form in drinking water.

5.2.3 Formation of aldehydes by chlorine dioxide.

Chlorine dioxide does not oxidize amino acids to aldehydes under conditions relevant to drinking water treatment and appears to be an oxidant of choice if the odour problem is to be minimized. However, during chlorine-based generation of chlorine dioxide, chlorine is formed at various quantities. For example, concentrations of free chlorine (expressed as a percentage of chlorine dioxide) monitored at the City of Edmonton Rossdale water treatment Plant have usually reached the levels between 5 to 20%, ocassionally exiding 100%. To avoid aldehyde production from reactions of chlorine with amino acids during disinfection with chlorine dioxide, the degree of chlorine contamination must be strictly limited.

5.3 Significance of amino acid levels in water supplies.

Odour problem associated with conversion of amino acids to odorous aldehydes upon chlorination or chloramination is likely to occur wherever surface water is utilized as a raw water source. This problem appears to be transient and may arise in water treatment plants which experience appropriate amino acids loading in their raw water.

Amino acids are common constituents of raw waters. Although free amino acids normally occur in such waters in low concentrations, bound amino acids, for example as proteins and peptides, can be more predominant. The levels of amino acids encountered in water will depend upon the degree of eutrophication and environmental stress. Evidence provided by Thurman (1986) suggests that many circumstances leading to increasing TOC levels will also result in an increase of amino acid levels. Commonly, surface run-off, especially rural run-off which carries decomposing organic matter collected on land may result in a substantial increase of amino acid concentrations. Intensive biological activity can also result in higher levels of amino acids released to the environment because of

bacterial metabolism and cell lysis. The study of free amino acids in oligotrophic waters such as those of mountain stream water in the Marmot Creak system in Alberta show the presence of total free amino acids in concentrations up to 2 µg/L (Telang et al., 1982). In contrast, eutrophic waters of Lake Mendota, contained 20 µg/L of individual amino acids (Gardner and Lee, 1973). Concentrations of total amino acids as high as 1000 µg/L for rivers and 6000 µg/L for lakes have been reported (Thurman, 1986).

Of particular interest is the finding of Le Cloirec and Martin (1985) that free amino acids can increase substantially during water treatment. They suggest that partial hydrolysis/depolymerization of proteins and peptides upon prechlorination along with biological activity, particularly during the filtration step, are involved. As indicated by these authors, amino acids are not removed during water treatment and become available for oxidation by a disinfectant.

These findings, along with the realization that amino acids may be produced by any biological activity in the distribution system, indicate that preventing the production of odorous aldehydes poses a difficult problem.

5.4 Control and treatment of odour.

Because the aldehydes are readily formed by disinfection reactions, it may be necessary to explore removing amino acids before undesirable reactions with the oxidants can occur.

Understanding the behaviour of amino acids in water treatment processes and investigating methods for their removal needs to be considered. The optimization of coagulation/flocculation processes with an aim of removing amino acids may be one such approach. Maximizing the removal of organic material in general will maximize the biological stability of treated water for downstream biological activity. As well, disinfectant demand can be minimized allowing lower disinfectant dosages hence lowering possible oxidant/amino acids ratios.

Ozone applications may be another possibility to be considered. In these experiments, ozone did not yield ssignificant quantities of aldehydes under drinking water conditions. Based on results obtained from ozonation of alanine which showed that ozone converted all the organic nitrogen to ammonia and nitrate Rice and Gomez-Taylor (1986) suggest that preozonation of raw water supplies could be used to destroy amino acids, prior to chlorination or chloramination.

Removal of odorous aldehydes which have formed in treatment processes as a means of treatment needs to be evaluated. One possibility, following from the observation on the reactions of ozone with aldehydes is that ozonation of water could be used to oxidize the odorous aldehydes. Van Hoof et al. (1986) studied the influence of coagulation, double layer filtration, storage and activated carbon filtration on aldehyde removal. The removal of the low molecular weight aldehydes by coagulation was poor as would be expected. Filtration was effective in decreasing aldehyde levels at temperatures above 10°C. Lower aldehyde levels were found in waters after storage and no measurable amounts of low molecular weight aldehydes were detected in the effluent of the activated carbon filters (van Hoof et al., 1986). Activated carbon was found to be effective in removing odour caused by aldehydes from drinking water during the odour event in Edmonton (Hrudey, 1986). However, activated carbon will not prevent the formation of aldehydes downstream of the point of its application i.e. in the distribution system.

Amino acids are ubiquitous in surface waters, therefore the problems which occurred in Edmonton are likely to have happened elsewhere without being recognized as such. Failure to recognize these specific compounds as the source of taste and odour problem is likely because their odour character is somewhat non-descript and they seem to stimulate widely differing responses from different individuals. Furthermore, in some cases, a large proportion of a sensory panel has been found to be anosmic to one of the aldehydes. These sensory recognition problems are further confounded by

analytical difficulties including the reality that routine GC/MS scanning is likely to be insensitive or blind to these compounds.

6. CONCLUSIONS

6.1 Experimental findings.

1. Under conditions relevant to water treatment, the amino acids valine, leucine, isoleucine and phenylalanine react with chlorine, monochloramine and ozone to form odorous aldehydes: isobutyraldehyde, isovaleraldehyde, 2-methyl butyraldehyde and phenylacetaldehyde, respectively.

2. Chlorine dioxide does not produce aldehydes in any substantial quantity under water treatment conditions. This finding probably occurs because chlorine dioxide does not form chlorinated amino acids which are intermediates in the formation of aldehydes when chlorination or monochloramination are used.

3. Chlorine is the most reactive with respect to aldehyde formation. The extent of aldehyde production depends upon temperature, relative ratio of chlorine to amino acids and reaction time. No significant effect of pH, ranging from 6 to 10, was observed.

4. In comparison to chlorine, monochloramine requires much longer reaction time for substantial conversion of amino acids to aldehydes. The most important factors affecting the reaction are temperature and pH, provided adequate ratios of monochloramine to amino acids are used.

5. Ozone yields very low levels of the specific aldehydes. Ozone oxidation is apparently less specific than that of chlorine or monochloramine which proceeds through the formation of chlorinated amino acids. As well, aldehydes produced during the course of reaction may be subsequently oxidized by the residual ozone.

6. The odorous aldehydes should be commonly present in amino acid-rich waters subjected to chlorination or chloramination processes. Identification of isobutyraldehyde, 2-methyl butyraldehyde and isovaleraldehyde in swimming pool water satisfied the foregoing expectation.

7. The presence of the odorous aldehydes in swimming pool samples at concentrations well above their respective threshold odour levels suggests that these aldehydes may be significant components of the commonly recognized "swimming pool" odour.

6.2 Implications for water treatment.

1. Amino acids are unavoidable dissolved organic species in surface water supplies or in waters harbouring biological activity, including filter beds and distribution system. Amino acids are not removed during conventional water treatment and can be present at the final disinfection step. Hence, they will be available for conversion to odorous aldehydes.

2. The odorous aldehydes possess very low threshold odour levels. When present in sufficient quantities, they will likely cause or contribute to taste and odour problems. Such incidents will be difficult to recognize because of the non-descriptive odour produced and the difficulty in identifying these aldehydes by routine analysis.

3. Conventional water treatment processes such as coagulation and filtration do not remove aldehydes present in waters. Activated carbon has been found to be effective in eliminating low molecular weight aldehydes.

4. Although chlorine dioxide does not react with amino acids producing odorous aldehydes, when used as a disinfecting agent, it usually contains some chlorine as a side product of chlorine dioxide generation. To reduce the possibility of taste and odour problem

arising from aldehyde production by reaction with chlorine, the degree of chlorine contamination must be strictly limited.

5. Because of very low yields of aldehydes formed during ozonation of amino acids and subsequent oxidation of aldehydes by residual ozone, sufficient levels of aldehydes that would result in odour problems are unlikely to form upon ozonation of drinking water containing the precursor amino acids.

6. Preferentially, control of aldehyde formation should be pursued rather than the removal of the odorous products. In consideration of this it is essential that levels of amino acids (whether free or in peptides) be reduced prior to chlorination or chloramination to prevent undesirable reaction leading to aldehyde formation.

7. Ozone produces only minute quantities of aldehydes from amino acids although it decomposes many amino acids and peptides. This suggests that preozonation of raw water supplies could be used to destroy amino acids prior to chlorination of chloramination.

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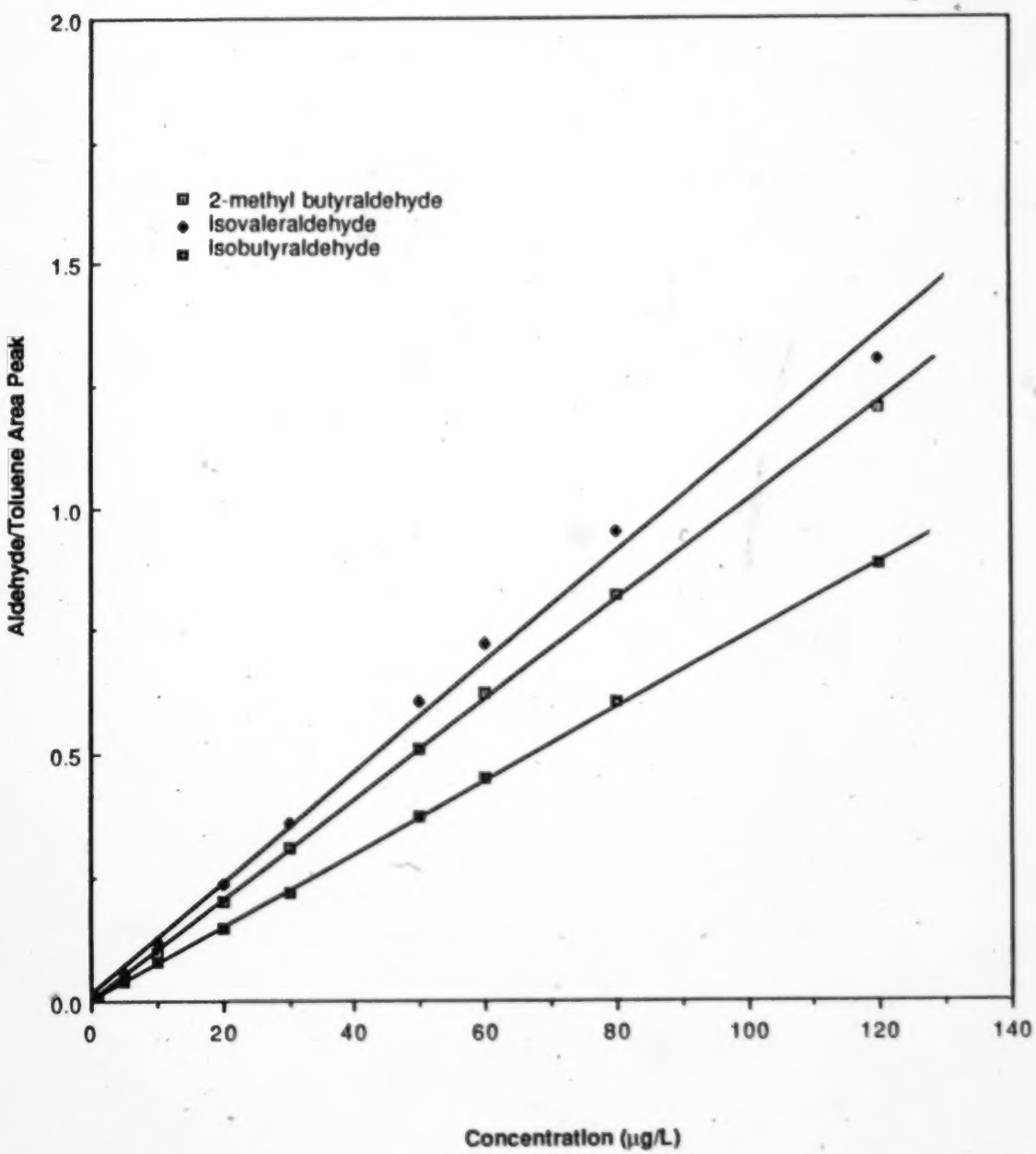
APPENDIX A

CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE ODOROUS ALDEHYDES

NAME	FORMULA	MOL. WEIGHT	BOILING POINT	MELTING POINT	DENSITY
Isobutyraldehyde	C_4H_8O	72.10	64°C	-65.9°C	0.794
Isovaleraldehyde	$C_5H_{10}O$	86.13	92°C	-51°C	0.785
2-methylbutyraldehyde	$C_5H_{10}O$	86.13	92°C	-50°C	0.803
Phenylacetaldehyde	C_8H_8O	120.16	195°C	33°C	1.027

APPENDIX B.1

Calibration curves for isobutyraldehyde, 2-methyl butyraldehyde and isovaleraldehyde.



LEAST SQUERS ESTIMATION OF THE SLOPE OF ISOVALERALDEHYDE STANDARD CURVE

	CONCENTRATION (µg/L) (y)								
	0.5	5	10	20	30	50	60	80	120
Aldehyde/Toluene peak area	0.0076	0.0755	0.151	0.302	0.435	0.755	0.906	1.208	1.812
	0.0071	0.0721	0.151	0.312	0.438	0.768	0.925	1.218	1.820
	0.0080	0.0791	0.148	0.301	0.428	0.721	0.880	1.202	1.848
	0.0073	0.0752	0.157	0.29	0.450	0.770	0.908	1.200	1.800
average	0.0075	0.0755	0.152	0.301	0.438	0.752	.0.904	1.207	1.820
std. dev.	0.0004	0.0029	0.004	0.009	0.009	0.021	0.019	0.008	0.020
rel. std. dev. (%)	5.1	3.8	2.5	3	2.1	2.8	2.1	0.7	1.1
Overall method precision	2.60%								

Precision of the estimate:

slope = $\Sigma y\bar{x}/\Sigma y^2$=427.83/28325=0.0151

variance(slope) = $\Sigma(\bar{x}-x)^2/n-1$=0.000039; S.E.(slope) = $\sqrt{s^2/\Sigma y^2}$=0.000037

95% C.I. for the estimate: slope ± S.E.*t(0.025;8) t(0.025;8)=2.306

95% C.I. (0.01501-0.01519)

LEAST SQUERS ESTIMATION OF THE SLOPE OF ISOBUTYRALDEHYDE STANDARD CURVE

	CONCENTRATION (µg/L) (y)								
	0.5	5	10	20	30	50	60	80	120
Aldehyde/Toluene peak area	0.0025	0.0025	0.050	0.101	0.150	0.250	0.318	0.400	0.600
	0.0028	0.0271	0.052	0.115	0.153	0.218	0.300	0.425	0.612
	0.0030	0.0225	0.049	0.098	0.147	0.233	0.330	0.405	0.625
	0.0020	0.0248	0.054	0.099	0.160	0.264	0.315	0.380	0.570
average	0.0026	0.0249	0.051	0.103	0.153	0.241	0.316	0.403	0.602
std. dev.	0.0004	0.0019	0.022	0.008	0.006	0.020	0.012	0.019	0.024
rel. std. dev. (%)	16.9	7.6	4.3	7.8	3.6	8.3	3.9	4.6	3.9
Overall method precision		6.80%							

Precision of the estimate:

slope = $\Sigma yx/\Sigma y$=142.7/28325=0.0050

variance(slope) = $\Sigma(x-x)/n-1$=0.000044; S.E.(slope) = $\sqrt{s}/\Sigma y$=0.0000395

95% C.I. for the estimate: slope ± S.E.*t(0.025;8) t(0.025;8)=2.306)

95% C.I. (0.00491-0.00509)

LEAST SQUERS ESTIMATION OF THE SLOPE OF 2-METHYLBUTYRALDEHYDE STANDARD CURVE

	CONCENTRATION (µg/L) (y)								
	0.5	5	10	20	30	50	60	80	120
Aldehyde/Toluene peak area	0.0061	0.0605	0.121	0.242	0.363	0.605	0.726	0.968	1.452
	0.0060	0.0565	0.128	0.199	0.311	0.590	0.698	0.925	1.430
	0.0058	0.0611	0.118	0.26	0.390	0.579	0.700	1.025	1.398
	0.0062	0.0582	0.130	0.241	0.371	0.639	0.790	0.968	1.482
average	0.0060	0.0591	0.124	0.236	0.359	0.603	0.729	0.972	1.441
std. dev.	0.0002	0.0021	0.006	0.0259	0.034	0.026	0.043	0.041	0.036
rel. std. dev. (%)	2.7	3.6	4.6	11	9.4	4.3	5.9	4.2	2.5
Overall method precision		5.40%							

Precision of the estimate:

slope = $\Sigma y\bar{x}/\Sigma y^2$=341.47/28325=0.0121

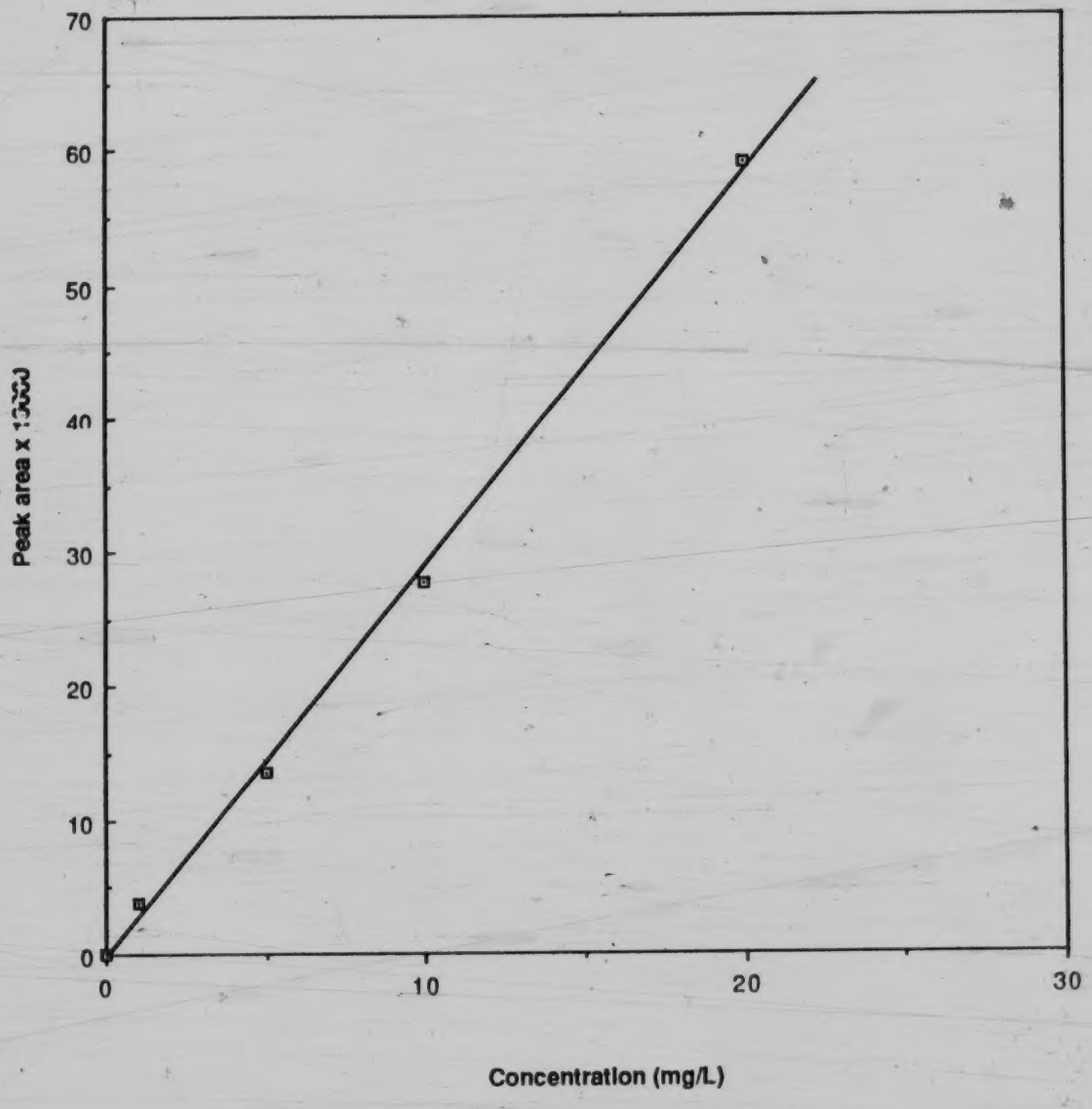
variance(slope) = $\Sigma(\bar{x}-x)^2/n-1$=0.0142/8=0.0018; S.E.(slope)=$\sqrt{s^2/\Sigma y^2}$=0.0003

95% C.I. for the estimate: slope ± S.E.*t(0.025;8) t(0.025;8)=2.306

95% C.I. (0.0118-0.0124)

APPENDIX B.2

Calibration curve for phenylacetaldehyde.



LEAST SQUERS ESTIMATION OF THE SLOPE OF PHENYLACETALDEHYDE STANDARD CURVE

	CONCENTRATION (mg/L) (y)			
	1	5	10	20
Aldehyde peak area	31714	136711	256030	624011
	28522	154734	278147	631073
	38844	135210	290947	612582
average	33027	142218	275041	622555
std. dev.	5285	10865	17664	9331
rel. std. dev. (%)	16.0	7.6	6.4	1.5
Overall method precision		7.9%		

Precision of the estimate:

slope = $\Sigma y\bar{x}/\Sigma y^2$=15945627/526=30315

variance(slope) = $\Sigma(\bar{x}-x)^2/n-1$=383058123;

S.E.(slope) = $\sqrt{s^2/\Sigma y^2}$=853.4

95% C.I. for the estimate: slope ± S.E.*t(0.025;3)

t(0.025;3)=3.182

95% C.I. (27600-33030)

APPENDIX C.1

ANALYSIS OF VARIANCE

RESULTS (µg/L):

	pH = 6	pH = 7	pH = 8	pH = 9	pH = 10
	88.4	80	75.5	84.4	72.5
	76.2	73.2	83.7	102	76.5
	79.2	68.1	78.7	84.4	68.8
	78.2	88	71.6	86.1	62
		72.5		80.5	
		92.2		87.4	
		86		95.3	
		81		89.1	
average	80.5	80.1	77.4	88.7	70.0
std. dev.	5.4	8.4	5.1	6.9	6.2
n	4	8	4	8	4
S.E.	2.7	3	2.6	2.4	3.1
95% C.L.	72≤µ≤89	73≤µ≤87	69≤µ≤86	83≤µ≤95	60≤µ≤80
C.V.	0.068	0.105	0.066	0.078	0.089

each set of data was checked for an outlier (ref. Davies, 1954)

ANOVA

SOURCE OF VARIATION	SS	d.f.	MEAN SS	F RATIO
BETWEEN SAMPLES	1019.1043	4	254.7761	5.2887
WITHIN SAMPLES	1108.0025	23	48.174	
TOTAL	2124.1068			

F(4,23;0.01)=4.26

F(calculated)=5.2887

F(4,23;0.01)<F(calculated) ; population means are not equal

(ref. Sokal and Rohlf, 1981)

APPENDIX C.2

FACTORIAL DESIGN-CHLORINE/LEUCINE REACTION

LEVELS	-	+
Time (min.)	30	120
Temp. (C)	4	20
pH	7	9

ESTIMATION OF VARIANCE

			Results (µg/L):				
Time	Temp.	pH	A	B	Average	A-B	$s^2=(A-B)^2/2$
-	-	-	5.9	4.6	5.3	1.3	0.8
+	-	-	16.1	15.1	15.6	1.0	0.5
-	+	-	65.2	50.8	58.0	14.4	103.1
+	+	-	83.6	73.3	78.5	10.3	52.7
-	-	+	16.6	11.3	13.9	5.3	14.0
+	-	+	30.6	21.9	26.3	8.8	38.3
-	+	+	80.6	73.2	76.9	7.4	27.7
+	+	+	95.5	80.4	88.0	15.1	114.1
						Total:	351.1
						variance =	43.9
						variance(effect) =	11.0
						std. error =	3.3

(ref. Box, Hunter and Hunter, 1978)

CALCULATIONS OF THE EFFECTS

							results:								
A	B	C	AB	AC	BC	ABC	a	b	average	A	B	C	AB	AC	BC	ABC
-1	-1	-1	1	1	1	-1	5.9	4.6	5.3	-5.3	-5.3	-5.3	5.3	5.3	5.3	-5.3
1	-1	-1	-1	-1	1	1	16.1	15.1	15.6	15.6	-15.6	-15.6	-15.6	-15.6	15.6	15.6
-1	1	-1	-1	1	-1	1	65.2	50.8	58.0	-58.0	58.0	-58.0	-58.0	58.0	-58.0	58.0
1	1	-1	1	-1	-1	-1	83.6	73.3	78.5	78.5	78.5	-78.5	78.5	-78.5	-78.5	-78.5
-1	-1	1	1	-1	-1	1	16.6	11.3	13.9	-13.9	-13.9	13.9	13.9	-13.9	-13.9	13.9
1	-1	1	-1	1	-1	-1	30.6	21.9	26.3	26.3	-26.3	26.3	-26.3	26.3	-26.3	-26.3
-1	1	1	-1	-1	1	-1	80.6	73.2	76.9	-76.9	76.9	76.9	-76.9	-76.9	76.9	-76.9
1	1	1	1	1	1	1	95.5	80.4	88.0	88.0	88.0	88.0	88.0	88.0	88.0	88.0
									SUM=	54.3	240.2	47.7	8.9	-7.4	9.1	-11.4
									SUM/4=	13.6	60.1	11.9	2.2	-1.9	2.3	-2.8

A = TIME
B = TEMPERATURE
C = pH

CRITERIA FOR DETERMINING SIGNIFICANT EFFECTS

95% C.I. = ß± [t(0.025;n-p)*S.E.(ß)]

EFFECT(µg/L)	ß	S.E.(ß)	C.I.
TIME	13.6	3.3	6.1-21.1
TEMP.	60.1	3.3	52.6-67.7
pH	12	3.3	4.5-1935
TIMExTEMP.	2.2	3.3	-5.3-9.7
TIMExpH	-1.9	3.3	-9.4-5.6
TEMP.xpH	2.3	3.3	-5.2-9.8
TIMExTEMP.xpH	-2.85	3.3	-10.3-4.6

t(0.025;16-7)=2.262

APPENDIX C.3

FACTORIAL DESIGN- MONOCHLORAMINE/LEUCINE REACTION

LEVELS	-	+
A. pH	7	10
B. monochloramine/leucine molar ratio	5	10
C. Temperature (C)	4	20
D. Time (h)	5	24

ESTIMATION OF VARIENCE

				RESULTS (µg/L):				
A	B	C	D	a	b	average	a-b	s^2
-	-	-	-	1.0	0.8	0.9	0.1	0.0
+	-	-	-	1.1	1.3	1.2	-0.3	0.0
-	+	-	-	0.5	0.9	0.7	-0.4	0.1
+	+	-	-	1.1	1.4	1.2	-0.3	0.1
-	-	+	-	13.0	15.8	14.4	-2.8	4.0
+	-	+	-	3.8	3.1	3.4	0.7	0.3
-	+	+	-	15.1	12.4	13.8	2.7	3.6
+	+	+	-	3.6	4.5	4.0	-0.9	0.4
-	-	-	+	1.6	1.9	1.7	-0.3	0.0
+	-	-	+	1.5	1.7	1.6	-0.2	0.0
-	+	-	+	2.8	2.6	2.7	0.2	0.0
+	+	-	+	1.6	1.9	1.8	-0.3	0.0
-	-	+	+	45.5	53.0	49.3	-7.5	28.3
+	-	+	+	3.1	4.5	3.8	-1.4	1.0
-	+	+	+	56.8	48.6	52.7	8.2	33.8
+	+	+	+	4.1	3.5	3.8	0.6	0.2
							Total=	71.8
							variance=	4.5
							variance(effect)=	1.1
							standard error=	1.1

(ref. Box, Hunter and Hunter, 1978)

CALCULATIONS OF THE EFFECTS

A	B	C	D	AB	AC	AD	BC	BD	CD	ABC	ABD	ACD	BCD	ABCD	results	A	B
-1	-1	-1	-1	1	1	1	1	1	1	-1	-1	-1	-1	1	0.9	-0.9	-0.9
1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	-1	1	1.21	1.21	-1.2
-1	1	-1	-1	-1	1	1	-1	-1	1	1	1	-1	1	-1	0.71	-0.7	0.71
1	1	-1	-1	1	-1	-1	-1	-1	1	-1	-1	1	1	-1	1.24	1.24	1.24
-1	-1	1	-1	1	-1	1	-1	1	-1	1	-1	1	1	1	14.4	-14	-14
1	-1	1	-1	-1	1	-1	-1	1	-1	-1	1	-1	1	-1	3.44	3.44	-3.4
-1	1	1	-1	-1	-1	1	1	-1	-1	-1	1	1	-1	1	13.8	-14	13.8
1	1	1	-1	1	1	-1	1	-1	-1	1	-1	-1	-1	1	4.04	4.04	4.04
-1	-1	-1	1	1	1	-1	1	-1	-1	-1	1	1	1	-1	1.73	-1.7	-1.7
1	-1	-1	1	-1	-1	1	1	-1	-1	1	-1	-1	1	-1	1.62	1.62	-1.6
-1	1	-1	1	-1	1	-1	-1	1	-1	1	-1	1	-1	1	2.65	-2.7	2.65
1	1	-1	1	1	-1	1	-1	1	-1	-1	1	-1	-1	1	1.75	1.75	1.75
-1	-1	1	1	1	-1	-1	-1	-1	1	1	1	-1	-1	-1	49.3	-49	-49
1	-1	1	1	-1	1	1	-1	-1	-1	-1	-1	1	-1	1	3.8	3.8	-3.8
-1	1	1	1	-1	-1	-1	1	1	1	-1	-1	-1	1	-1	52.7	-53	52.7
1	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	3.8	3.8	3.8
															SUM=	-115	4.25
														EFFECT	= SUM/8	-14	0.53

A = pH
B = monochloramine/leucine molar ratio
C = Temperature
D = Time

C	D	AB	AC	AD	BC	BD	CD	ABC	ABD	ACD	BCD	ABCD
-0.9	-0.9	0.9	0.9	0.9	0.9	0.9	0.9	-0.9	-0.9	-0.9	-0.9	0.9
-1.2	-1.2	-1.2	-1.2	-1.2	1.21	1.21	1.21	1.21	1.21	1.21	-1.2	1.21
-0.7	-0.7	-0.7	0.71	0.71	-0.7	-0.7	0.71	0.71	0.71	-0.7	0.71	-0.7
-1.2	-1.2	1.24	-1.2	-1.2	-1.2	-1.2	1.24	-1.2	-1.2	1.24	1.24	-1.2
14.4	-14	14.4	-14	14.4	-14	14.4	-14	14.4	-14	14.4	14.4	14.4
3.44	-3.4	-3.4	3.44	-3.4	-3.4	3.44	-3.4	-3.4	3.44	-3.4	3.44	-3.4
13.8	-14	-14	-14	13.8	13.8	-14	-14	-14	13.8	13.8	-14	13.8
4.04	-4	4.04	4.04	-4	4.04	-4	-4	4.04	-4	-4	-4	4.04
-1.7	1.73	1.73	1.73	-1.7	1.73	-1.7	-1.7	-1.7	1.73	1.73	1.73	-1.7
-1.6	1.62	-1.6	-1.6	1.62	1.62	-1.6	-1.6	1.62	-1.6	-1.6	1.62	-1.6
-2.7	2.65	-2.7	2.65	-2.7	-2.7	2.65	-2.7	2.65	-2.7	2.65	-2.7	2.65
-1.8	1.75	1.75	-1.8	1.75	-1.8	1.75	-1.8	-1.8	1.75	-1.8	-1.8	1.75
49.3	49.3	49.3	-49	-49	-49	-49	49.3	49.3	49.3	-49	-49	-49
3.8	3.8	-3.8	3.8	3.8	-3.8	-3.8	3.8	-3.8	-3.8	3.8	-3.8	3.8
52.7	52.7	-53	-53	-53	52.7	52.7	52.7	-53	-53	-53	52.7	-53
3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	-3.8
133	77.6	-2.7	-115	-76	2.47	4.69	70.2	-1.6	-5.7	-72	2.27	-72
16.7	9.7	-0.3	-14	-9.4	0.31	0.59	8.78	-0.2	-0.7	-9	0.28	-9

CRITERIA FOR DETERMINING SIGNIFICANT EFFECTS

95% C.I. = ß± [t(0.025;n-p)*S.E.(ß)]

EFFECT(µg/L)	ß	S.E.(ß)	C.I.
pH	-14.4	0.7	-15.9 to -12.9
molar ratio	0.5	0.7	-1.0 to 2.0
temp.	16.7	0.7	15.2 to 18.2
time	9.7	0.7	8.2 to 11.2
pHxmolar ratio	-0.3	0.7	-1.8 to 1.2
pHxtemp.	-14.4	0.7	-15.9 to -12.9
pHxtime	-9.4	0.7	-10.9 to -7.9
molar ratioxtemp	0.3	0.7	-1.2 to 1.8
molar ratioxtime	0.6	0.7	-0.9 to 2.1
temp.xtime	8.8	0.7	7.3 to 10.3
pHxmolarratixtemp	-0.2	0.7	-1.7 to 1.3
pHxmolar ratioxtime	-0.7	0.7	-2.2 to 0.8
pHxtempxtime	-0.9	0.7	-10.5 to -7.5
molar ratioxtempxtime	0.3	0.7	-1.2 to 1.8
pHxmolar ratioxtempxtime	-9	0.7	-10.5 to -7.5

t(0.025;17)=2.11